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On the Identity of Haematoidin and Bilirubin

By Arnold R. Rich and John H. Bumstead

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In 1847 Virchow discovered in old haemorrhages a pigment which resembled bilirubin, the red-brown pigment of the bile (7). He was unable to satisfy himself that this pigment was identical with bilirubin, and he therefore gave it the name "haematoidin." He expressed the belief that the pigment was derived from the haemoglobin of disintegrated red blood cells. Many subsequent investigators have confirmed the observation that when red blood cells escape from the blood vessels into almost any tissue of the body, the pigment designated by Virchow as "haematoidin" may be formed from the haemoglobin of the disintegrating cells. Although Virchow did not prove the identity of haematoidin and bilirubin, his observation was the first important evidence in favor of the belief that bile pigment can be derived from haemoglobin.

Obviously, it is a matter of great importance in dealing with the subject of the metabolism of bile pigment and of haemoglobin to know whether or not haematoidin and bilirubin are actually one and the same substance; for the site of origin of bile pigment is still an unsolved problem, and if bilirubin itself can be formed from haemoglobin spilled into any tissue or from disintegrating red cells in a thrombus within a blood vessel, it becomes clear that bile pigment must be formed either extracellularly by some enzyme present *in the plasma and in all tissue fluids* or else by the activity of living cells. Up to the present there is no proof of the existence of an enzyme having the power to transform haemoglobin into bile pigment. If the pigment is formed intracellularly it would seem probable that the cells concerned with its formation are those of the reticulo-endothelial type; for these cells are present in the blood and in practically every tissue of the body; they accumulate in large numbers in and about blood extravasations, and they are well known to be specifically active in erythrophagocytosis and in the breaking down of haemoglobin into simpler substances.

The question of the identity of haematoidin and bilirubin has long been a subject of controversy. Although most writers have accepted the belief that bile pigment itself is formed at the site of haemorrhages, there have always been investigators

who have opposed this view because of numerous reports of the failure of haematoidin to give various reactions characteristic of bilirubin. For a discussion of the controversy up to the year 1900 one may consult the reviews of Legg (1) who opposed the belief that the two pigments are identical, and of Stadelmann (2) who accepted it. Since 1900, van den Bergh and Snapper (4) and others have shown that the pigment formed in haemorrhages gives a diazo-reaction characteristic of bilirubin, and Hooper and Whipple (3) have shown that it gives a positive Huppert test—a modification of the Gmelin test for bilirubin. Recently Fischer and Reindel (5) have reported studies on the similarity of the crystal forms of haematoidin and bilirubin and have stated that the percentage composition of haematoidin agrees closely with that of gall-bladder bilirubin. Unfortunately, the haematoidin analyzed was obtained from an echinococcus cyst of the liver, and since it is well known that such cysts may be in communication with bile-ducts, it is impossible to be sure that the substance which Fischer and Reindel studied was not actually bilirubin from the bile. The same objection must apply to the analysis made by Robin (6) from material obtained from similar cysts in the liver. Up to the present there has been no analysis of haematoidin obtained from haemorrhages outside the liver.

Among recent authoritative writers who hesitate to accept the identity of haematoidin and bilirubin may be mentioned Hueck (8), who states that although haematoidin appears to contain a substance resembling bilirubin, one is by no means justified in believing that bile pigment itself is formed at the site of blood extravasations; and Thannhauser (9), who believes that there are physical and chemical differences between bilirubin and haematoidin which are sufficient to disturb the acceptance of the identity of these substances. He states that their crystal forms are different and that haematoidin does not give the diazo-reaction characteristic of bilirubin.

Undoubtedly, one of the chief reasons for the uncertainty and the conflicting reports concerning the properties of haematoidin has been the fact that only very small amounts of this pigment have been available for study. The amount which can be obtained from old haemorrhages is usually very small, and up to the present no one has attempted to produce in experimental extravasations a quantity of the pigment sufficient to permit a complete examination of its properties. We have recently obtained an unusually plentiful yield of haematoidin from the haemorrhagic fluid contained in a very large cyst of the omentum removed at operation from a child by Dr. W. F. Rienhoff of the Surgical Service. This cyst was completely encapsulated, pedunculated, and was entirely free from communication with the bile-ducts, the liver, or any other organ. It contained about three litres of a dark brownish-red fluid. In this fluid were found great numbers of large cells which, on vital staining with neutral red, were found to have the characteristics of clasmatocytes. The majority of

these phagocytic cells were engorged with red blood cells in different stages of digestion. A van den Bergh test carried out on the hemorrhagic fluid contained in the cyst gave a reading equivalent to fifteen units of bilirubin. On the other hand, the bilirubin content of the child's plasma was unusually small —so small, indeed, that a quantitative estimation was not possible.

The haematoidin was extracted from the cyst fluid with warm chloroform. Abundant crystals obtained by evaporation of the chloroform in the air were dissolved in chloroform and recrystallized several times. Although no attempt was made to subject these crystals to minute crystallographic studies, it may be said that they occurred in the form of rhomboid and cigar-shaped plates and as sheaves of needles which were quite similar to the crystal forms obtained from gall-bladder bilirubin after recrystallization from chloroform.

This recrystallized haematoidin has been subjected to the following tests and reactions which are used for the identification of bilirubin, and in every instance the haematoidin behaved precisely as did a control of gall-bladder bilirubin.

1. Solubility. Both bilirubin and haematoidin are insoluble in water and in acid solutions. They are slightly soluble in ether, somewhat more so in 95 per cent alcohol, and are readily soluble in chloroform, in dilute alkali, in dimethylaniline and in carbon bisulphide, the solutions having a yellow or orange color depending upon the concentration.

Both bilirubin and haematoidin are precipitated from alkaline solutions by the addition of a sufficient amount of acid; they are carried down with the precipitate of calcium carbonate when calcium chloride and sodium carbonate are added to their alkaline solutions; they are also precipitated from alkaline solutions by the addition of barium sulphate or ammonium sulphate; and both pigments can be readily extracted from their chloroform solutions by shaking with dilute alkalies.

2. Spectroscopic. Neither bilirubin nor haematoidin exhibits any specific absorption bands, but both show an identical continuous absorption of the violet end of the spectrum.

3. Special tests characteristic of bilirubin. a. Gmelin test. When a few crystals of haematoidin are treated with a dilute solution of nitrous acid under the microscope, they can be seen to change in color to green, blue, violet, red and finally to reddish-yellow, successively. This is the play of colors given by bilirubin on similar treatment, and the order of appearance of the colors is the same when either pigment is used. The same play of colors occurs when a solution of either of these pigments is layered with nitrous acid.

b. Huppert's test. When an alkaline solution of haematoidin is treated with calcium chloride and sodium carbonate, the pigment is completely precipitated with the calcium carbonate. When this yellow precipitate is heated in acid alcohol the liquid becomes bright green. On the addition of a small amount of sodium nitrite the color changes to a clear robin's egg blue.

c. Obermeyer's test. Upon the addition of Obermeyer's reagent to a solution of haematoidin a green color appears.

d. Trichloracetic acid test. Crystalline haematoidin dissolves readily in melted trichloracetic acid with the production of a green color.

e. Further oxidation tests. When an alkaline solution of either bilirubin or haematoidin is allowed to stand exposed to the air the color changes in a short time from yellow to green. When a solution of either of these pigments is heated with a few drops of concentrated sulphuric acid and of formalin, the yellow color changes rapidly through green to blue. The green color in these tests probably represents the oxidation of bilirubin to biliverdin—the process which is responsible for the appearance of the green color in the first step of the Gmelin reaction.

f. Oxidation to cholecyanin. The blue-colored substance which appears during the progress of the Gmelin reaction has been studied especially by Jaffe, Stokvis and Heynsius and Campbell (10), and is termed "bilicyanin" or "cholecyanin." This substance can be identified by its characteristic absorption spectrum. It can be prepared from bilirubin by oxidation in a wide variety of ways. If the Gmelin test be carried out by layering dilute nitrous acid upon a chloroform solution of bilirubin and shaking occasionally, the progression of colors will occur slowly throughout the chloroform solution and can be retarded at any desired moment by the addition of an alkali or, indeed, by diluting the supernatant nitrous acid with water. When our haematoidin was subjected to the Gmelin test in this manner and examined spectroscopically at the stage when the solution became blue in color, it showed the absorption spectrum characteristic of cholecyanin in acid chloroform solution (10). We have also prepared cholecyanin from haematoidin by oxidation with bromine.

g. Rosin's iodine test. When an alcoholic solution of iodine is layered with a solution of bilirubin, a bright emerald ring appears at the point of contact of the two liquids. There has been some question as to whether the green substance formed is merely an oxidation product (biliverdin ?) or whether it represents a halogen substitution product. The emerald ring develops promptly when a solution of haematoidin is treated in this manner.

h. Halogen substitution. When a water-free chloroform solution of bilirubin is treated with bromine dissolved in chloroform, a precipitate is formed which dissolves in alkalies with the production of a deep blue color. This substance is said to be tribrombilirubin (11). When haematoidin is used instead of bilirubin the reaction proceeds exactly as in the case of bilirubin itself.

i. Ehrlich's diazo-reaction. When a solution of bilirubin is treated with Ehrlich's diazo-reagent, a characteristic reddish-violet color appears, due to the formation of azo-bilirubin. This reaction develops promptly only when the bilirubin is dissolved in alcohol. Haematoidin gives an identical color when similarly treated, and the promptness with which the reaction occurs is likewise influenced by the presence of alcohol. When

a solution of azo-bilirubin is layered with concentrated hydrochloric acid, a deep bluish-violet ring is formed between the two liquids; if it is layered with alkaline solutions, a series of rings which are red, yellowish, and finally green, from above downward, appear. The red solution resulting from the treatment of haematoidin with the diazo-reagent reacts in a manner exactly as does bilirubin when layered with acid or alkali.

Examined spectroscopically, solutions of azo-bilirubin and "azo-haematoidin" showed an identical absorption band beginning at a point just to the right of D, and extending to F.

TABLE 1

	BILIRUBIN	HAEMATOIDIN
Crystals	Rhomboid and cigar-shaped plates, and needles	Rhomboid and cigar-shaped plates, and needles
Spectroscopic examination	Continuous absorption of violet end	Continuous absorption of violet end
Solubility in water	0	0
Solubility in acid	0	0
Solubility in ether	Very slight	Very slight
Solubility in alcohol	Slight	Slight
Solubility in chloroform	Easily	Easily
Solubility in carbon bisulphide	Easily, with yellow color	Easily, with yellow color
Solubility in dimethylaniline	Easily	Easily
Solubility in alkali	Easily	Easily
Extraction from chloroform by alkali	+	+
Ppt. by acids from alkali	+	+
Ppt. by $BaSO_4$ and $(NH_4)_2SO_4$	+	+
Ppt. with calcium carbonate	+	+
Gmelin test for bilirubin	+	+
Huppert test for bilirubin	+	+
Obermeyer test for bilirubin	+	+
Alkaline solution standing in air	Turns green	Turns green
Heating with H_2SO_4 and CH_2O	Turns green, then blue	Turns green, then blue
Rosin's iodine test for bilirubin	+	+
Halogen substitution (tribrombilirubin test for bilirubin)	+	+
Ehrlich diazo-reaction for bilirubin (Indirect van den Bergh)	Red azo-derivative	Red azo-derivative
Red azo-derivative + strong acid	Bluish violet	Bluish violet
Red azo-derivative + strong alkali	Green	Green
Oxidation to cholecyanin with characteristic absorption spectrum	+	+
Reduction to urobilin (hydrobilirubin) with characteristic absorption spectrum	+	+

j. Reduction to urobilin (hydrobilirubin). When sodium amalgam is allowed to stand in contact with

solution of bilirubin, the bilirubin is reduced to "hydrobilirubin," which gives the absorption spectrum and characteristic reactions of urobilin. Haematoidin was subjected to this reaction in the following manner: Sodium amalgam was added to a solution of haematoidin in dilute alkali.[1] After standing over night the solution had become much lighter in color. A portion of this solution was treated with ammonia, a few drops of Lugol's solution, and a saturated alcoholic solution of zinc acetate. After centrifugalization, the supernatant fluid exhibited a very marked greenish fluorescence (Schlesinger's test for urobilin). When a few drops of a solution of p-dimethylamino-benzaldehyde in HCl were added to the reduced haematoidin solution a deep red color appeared, which became more intense on heating (Ehrlich's "aldehyd reaction" for urobilinogen). Spectroscopic examination of the reduced haematoidin solution, made acid by addition of HCl, showed an absorption band between b and F, overlying F. When the solution was made alkaline and zinc chloride added, the band was widened so that the left border lay between E and b. These absorption bands are well known to be those characteristic of urobilin or hydrobilirubin under similar conditions.

DISCUSSION

Scattered through the literature since Virchow's discovery of "haematoidin," there can be found conflicting statements concerning the physical and chemical properties of this pigment as compared with those of bilirubin. It has been said that haematoidin does not regularly give a typical Gmelin reaction (8); that the pigment dissolves in carbonbisulphide, yielding a red solution instead of the orange-yellow solution which bilirubin forms with this solvent (1); that haematoidin, in contrast to bilirubin, is soluble in ether and insoluble in alkalies (1); that haematoidin exhibits absorption bands spectroscopically which are not characteristic of bilirubin (1); and, very recently, that haematoidin does not give the Ehrlich diazo-reaction (9). Many of the studies carried out upon haematoidin have been made upon material extracted from old haemorrhages preserved in formalin or alcohol. It is known that the properties of even gall-bladder bilirubin may change upon mere standing in the air, and it seems probable that some of the conflicting observations in the literature may be explained as the results of changes in the haematoidin. Further, it seems certain that in some cases the observers were not dealing with haematoidin at all, but with lipoidal substances. This is particularly true of those who used extracts from corpora lutea.

In the present study we have found that haematoidin and bilirubin have similar crystal forms, the same solubility properties, yield the same oxidation and reduction products, form the same azo-combination and halogen substitution products, and behave in precisely the same manner in all of the chemical tests to which we

[1] We desire to express our thanks to Dr. W. A. Perlzweig for preparing the sodium amalgam and for his helpful advice.

have subjected them. Although neither exhibits any characteristic absorption bands spectroscopically other than continuous absorption of the violet end, identical substances exhibiting characteristic absorption spectra have been prepared from both by reduction (hydrobilirubin) and by oxidation (cholecyanin). As far as we have been able to find, this represents the first preparation from haematoidin of these characteristic oxidation and reduction products of bilirubin.

These facts make it appear most probable that the pigment "haematoidin" which is formed from haemoglobin at the site of blood extravasations is really bilirubin itself. Further evidence that this is true lies in the fact that in birds, in which biliverdin, an oxydation product of bilirubin, is the predominant pigment of the gall-bladder bile, biliverdin (i.e., a bright green pigment giving a characteristic Gmelin reaction beginning, of course, with green) is commonly formed in haemorrhages by the side of "haematoidin" crystals. We do not know the spatial formula of bilirubin itself, and until a successful comparison of the structure of bilirubin and haematoidin has been made, it is obvious that we cannot be sure that the two pigments are identical. However, most of the objections to the identification of haematoidin with bilirubin have come from writers who have hesitated to accept the belief that bilirubin can be formed extrahepatically. In the light of our present certainty that the extrahepatic formation of bilirubin can occur, the results of the above comparison of the properties of haematoidin with those of bilirubin make it appear unnecessary to continue to distinguish between the pigment which is formed when haemoglobin is broken down in the tissues and that formed when haemoglobin is broken down after being liberated into the blood stream. At least it would seem that the burden of proof must rest upon those who reject the identity of bilirubin and haematoidin. The common statement that haematoidin is an isomer of bilirubin rests upon no evidence of any sort.

SUMMARY

Haematoidin obtained from the haemorrhagic contents of a cyst of the omentum has been subjected to the physical and chemical tests and reactions which are characteristic of bilirubin, and in every instance it has behaved precisely as did a control of pure bilirubin.

In this study urobilin (hydrobilirubin) has been prepared from haematoidin by reduction, and cholecyanin (bilicyanin) by oxidation. These are characteristic oxidation and reduction products of bilirubin.

These facts are offered in support of the belief that haematoidin and bilirubin are identical.

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On the Extrahepatic Formation of Bile Pigment

By Arnold Rice Rich

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THE liver-extirpation experiments of Minkowski and Naunyn in 1886 led to a quite universal acceptance of the dictum "Ohne Leber kein Ikterus," for the marked jaundice which regularly followed intravascular haemolysis in normal animals (birds) failed to make its appearance if the liver were removed before haemolysis was induced (1). McNee (2) repeated these experiments in 1913 with essentially the same result but interpreted this result, as is well known, in a manner quite different from that of Minkowski and Naunyn. In the same year Whipple and Hooper investigated the question of the site of origin of bile pigment, using the dog as the experimental animal. They did not remove the liver from the body, but sought to exclude it from the circulation by means of certain ligatures before introducing haemoglobin into the blood stream. Contrary to the results of Minkowski and Naunyn and of McNee, they found that haemoglobin circulating, supposedly, through the head and thorax and entirely outside the liver, was converted into bile pigment within two hours in the blood stream, and that an actual tissue jaundice appeared (3). The writer has since shown that the method of ligaturing used by Whipple and Hooper does not actually shut off all of the blood supply to the liver (4). Under the conditions of their experiments, blood circulates through the liver, and the Kupffer cells remain alive and actively phagocytize foreign particles carried to them by the circulation which persists after ligation of all of the vessels assumed by Whipple and Hooper to be necessary to exclude the liver completely from the circulation. It became clear, therefore, that the work of Whipple and Hooper could no longer be regarded as having proved the extrahepatic origin of the bile pigment which was formed in their experiments. Recently, Makino (6) has repeated Whipple and Hooper's experiments and has confirmed the writer's observation by finding microscopic evidence that haemoglobin introduced into the blood stream circulating, supposedly, entirely outside the liver, does find its way into that organ, is phagocytized by the Kupffer cells and is broken down there into its iron-containing by-product, haemosiderin. Whipple,

himself, believes that the Kupffer cells have the power to convert haemoglobin into bile pigment (5), and, indeed, bilirubin, the iron-free by-product of the disintegration of haemoglobin, did appear in the plasma in these experiments. This work is a further proof that any bilirubin found in the blood-stream of such an animal as those in Whipple and Hooper's so-called "head-thorax circulation" experiments cannot be accepted as having been formed outside the liver. It is obvious, as Makino insists, and as the writer has carefully pointed out (4), that only a small portion of the normal blood supply to the liver reaches the organ through these persisting vessels. However, no correct estimate of the amount of blood which can enter and leave the liver under the conditions of Whipple and Hooper's experiments can be gained without the injection of some easily recognizable substance (such as india ink) into the circulation, and this experiment Makino did not make. Aschoff, in whose laboratory Makino's experiments were carried out, has recently written, in a discussion of the work of Makino and of Rich: "the amount of pigment (haemoglobin) which enters the liver in this manner is relatively small. However, these blood-vessel connections could suffice to explain the formation of bile pigment in the experiments of Whipple and Hooper" (7).

In other experiments the writer found that when the liver and all the other abdominal viscera were removed from the body, or else actually excluded from the circulation by a different method of ligation, no bile pigment whatever was formed from haemoglobin circulating through the head and thorax even within a period several times as long as that in which Whipple and Hooper, with incomplete exclusion of the liver, had observed a distinct tissue jaundice. The test for bilirubin in these experiments was much more delicate than the one used by Whipple and Hooper, the operation devised for excluding the liver from the circulation by ligatures was much less severe than their intrathoracic operation requiring continuous artificial respiration throughout the experiment, and half of the animals of the series lived longer than the time found necessary by Whipple and Hooper for the appearance of a canary-yellow tissue jaundice (4); yet not even a trace of bile-pigment could ever be detected in the plasma of any of the animals. These facts, together with the demonstration that Whipple and Hooper had not really excluded the liver from the circulation in their experiments, seemed to justify the conclusion that the experiments of Minkowski and Naunyn and of McNee, which had emphasized the importance of the liver as a normal site of origin of bile pigment, were substantiated. Shortly after the appearance of this work, McNee and Prusik published in detail further experiments on this point (which had already been reported briefly several months before (10)) with the conclusion that "haemoglobin is not rapidly changed into bile pigment within the circulating blood when the liver has been excluded and a circulation maintained only in the head and thorax. This supports the

results arrived at by Rich and is against the view of Whipple and Hooper. The site of origin of bile pigment in haemolytic jaundice must therefore be considered, for the present, as undetermined" (8).

However, Mann had devised a method by which the liver can be removed from a dog without interfering with the circulation of the rest of the body, and at the December, 1923, meeting of the American Physiological Society, Mann, Bollman and Magath (9) reported that in such hepatectomized animals a gradual appearance of bile pigment in the plasma and urine was regularly observed. As the following experiments will show, I can entirely confirm the very important demonstration of these investigators that the formation of bilirubin can proceed in the complete absence of the liver.

It was a matter of some concern to me to try to understand why bilirubin made its appearance in the plasma of their dogs but had failed to appear in my animals or in McNee's restricted circulation experiments. Since, in the experiments of Mann, Bollman and Magath, the pigment made its appearance even in eviscerated animals, the only important differences in technique between their experiments and those of the writer or of McNee were (1) the fact that the circulation of the musculo-skeletal structures of the entire animal was left intact in the former experiments, whereas in the latter the circulation was restricted either to the head and thorax or to a "heart-lung circulation" preparation; and (2) the fact that the method for removal of the liver devised by Mann and his associates involves a great reduction of the blood supply to the liver for a period of days or weeks before the organ is extirpated. Now, it is well known that there is an intimate relationship between the spleen, the liver and the bone-marrow in so far as they are concerned with haemoglobin metabolism, and one is familiar with examples of an interchange of their functions when abnormal conditions demand it. Thus, although the bone-marrow alone in normal adult life manufactures the haemoglobin-containing red cells, an abnormal reduction in the amount of functioning marrow is often followed by a compensatory formation of red cells in the liver or the spleen. Likewise, phagocytosis of red cells or of their fragments is a prominent activity of the spleen especially in certain animals (including the dog), but there is evidence that this function may be taken over by the phagocytic cells of the liver, lymph glands and bone-marrow following splenectomy (11, 12). Assuming that bilirubin, a katabolic product of haemoglobin, were normally formed in the liver, as the experiments of Minkowski and Naunyn, McNee, and Rich indicated, it seemed possible that in the experiments of Mann, Bollman and Magath the prolonged reduction of the blood supply to the liver might have created the necessity for a compensatory reaction similar to those described above, with the result that the spleen and bone-marrow might have been stimulated to take over the function of breaking down haemoglobin into bile pigment. Rosenthal (13) has raised just this ob-

jection to Mann's method of hepatectomy in another connection; and, in my above-mentioned paper, I had submitted the possibility that, although the liver appeared to be the normal site of origin of bile pigment, the spleen and bone-marrow might assume that function in time of need as a compensatory reaction (4). Furthermore, it has been the experience of numerous investigators (14, 12, 15) that the formation of bile pigment is seriously disturbed after the ligation of the portal vein in the Eck-fistula, and the method of hepatectomy used by Mann, Bollman and Magath involves a similar reduction of the hepatic blood supply during a period of time before the liver is removed. The present experiments were undertaken, therefore in the attempt to learn whether there exists in the circulation outside the liver a ready mechanism for the conversion of haemoglobin into bile pigment or whether the bilirubin formed in the experiments of Mann and his coworkers represented the result of an abnormal extrahepatic reaction to compensate for the decreased liver function which must result from a protracted reduction of the hepatic blood supply. The experiments of McNee and of the writer indicated that the sudden removal of a normal liver left no important mechanism by which circulating haemoglobin could be rapidly changed into bilirubin; but in their experiments only half, or less, of the entire musculo-skeletal system was left with an active circulation. The question which presented itself, therefore, was: Will bile pigment be formed promptly from circulating haemoglobin if the liver is removed, without any previous interference with its function, from animals in which the entire musculo-skeletal system is left within the circulation?

EXPERIMENTS[1]

I. Circulation maintained throughout entire musculo-skeletal system; hepatectomy and evisceration without previous restriction of hepatic circulation.

Since it is absolutely necessary to ligate the vena cava below the liver in order completely to remove that organ from dogs, I had, in my previous experiments, ligated the aorta below the diaphragm in order to prevent congestion of the parts behind the vena cava ligature (4). This resulted in a considerable restriction of the circulation. In the present experiments venous drainage for the hind parts after hepatectomy was provided by ligating the vena cava just below the renal veins and the ovarian (or spermatic) vein at its entrance to the left renal vein several weeks before hepatectomy was performed. In this manner it was intended to induce the development of a collateral circulation for the parts behind the vena cava ligature, so that when the liver was subsequently removed the aorta could be left untied, and the conditions of the experiments of Mann, Bollman and Magath would be reproduced in all details save for the fact that, in these experiments, the circulation of the liver was interfered with at no time before hepatectomy.

[1] Experiments reported before the Philadelphia Pathological Society, March, 1924.

The sudden ligation of the vena cava had only a very transient effect upon most of the animals. Usually, during several hours following the operation, there was an evident weakness of the hind legs which in two cases resulted in an inability to move them. In these instances the weakness was accompanied by muscle-twitchings. But after twelve to twenty-four hours the animals could run about with ease. In spite of the apparently great hindrance to the venous outflow, pitting oedema was observed only in two males and in none of the six females so treated. Both of these males developed marked oedema of the legs, lower abdomen and genitals. One of them died within twenty-four hours. At autopsy no thrombus was found in the vena cava or large veins and there was no evidence whatever of infection. The oedema of the other animal disappeared after about two weeks. This animal died later from distemper. At autopsy there was no evidence that there had been any infection of the operative site or of the large veins. In most cases the veins of the abdominal wall became distinctly but not markedly enlarged several weeks after the ligation.

Expt. I. Dog 8. Female. Weight 15 pounds.

January 26, 1924: Morphia and ether anesthesia. Vena cava ligated between right ovarian vein and renal veins. Left ovarian vein ligated at entrance to renal vein. Six hours later the animal stands unwillingly and walks rather stiffly.

January 27, 1924: Uses hind legs easily. Can be made to run about without apparent abnormal fatigue of the hind legs. There is no edema whatever.

February 19, 1924: Weight 16¼ pounds. Excellent condition.

Morphia and ether anesthesia. Abdomen opened in midline. Coeliac axis, mesenteric arteries and the renal vessels ligated. No ligature is placed about the aorta itself. All structures at hilum of liver cut between mass ligatures. Ligation of oesophagus just below diaphragm. Removal of stomach, intestines, pancreas, spleen and kidneys. Vena cava ligated between liver and diaphragm. Liver cut free and removed intact from the body. Bleeding from diaphragmatic stump controlled by purse-string suture. Abdomen packed with warm towels and closed. Operation completed at 11:10 a.m. Animal in good condition. 11:20 a.m., 8 cc. blood taken from jugular vein for test (Spec. I). 11:30 a.m., given 25 cc. 20 per cent glucose intravenously. 12:30 p.m., 8 cc. blood taken (Spec. II). Condition excellent. Good pulse in femoral arteries. 1 p.m., 8 cc. blood taken (Spec. III); 1:30 p.m., 20 cc. 20 per cent glucose intravenously. 2 p.m., 8 cc. blood taken (Spec. IV). 2:30 p.m., 8 cc. blood taken (Spec. V). 2:40 p.m., struggles violently and ¼ gr. morphia given. 3:10 p.m., 8 cc. blood taken (Spec. VI). 3:30 p.m., 8 cc. blood taken (Spec. VII); 20 cc. 20 per cent glucose intravenously. Femoral pulse strong on both sides. 4 p.m., condition excellent. 8 cc. blood taken (Spec. VIII). 4:30 p.m., 8 cc. blood taken (Spec. IX). 4:45 p.m., gasps for breath; heart stops.

Autopsy: Performed at once. The sub cutaneous and epicardial fat is normally pale in color. 10 grams of this fat give negative tests for bile pigment. The heart and lungs are normal. The liver has been completely removed. There is no free blood in the peritoneal cavity. The ligatures are intact. The old vena cava ligature completely occludes the vessel. The aorta and the iliac and femoral arteries are patent. A cannula is inserted into a small vein just above the foot and a concentrated solution of Berlin blue is injected under very moderate pressure. In a short while the blue color appears in the right side of the heart, showing that there is a ready venous return from the legs in spite of the ligated vena cava.

Bilirubin tests on the specimens of blood taken during the experiment

NUMBER OF SPECIMEN	TIME AFTER REMOVAL OF LIVER	VAN DEN BERGH TEST (INDIRECT)
I	10 min.	Negative
II	1 hr. 10 min.	Negative
III	1 hr. 50 min.	Negative
IV	2 hrs. 50 min.	Negative
V	3 hrs. 20 min.	Negative
VI	4 hrs.	Faintly positive
VII	4 hrs. 20 min.	Faintly positive
VIII	4 hrs. 50 min.	More strongly positive
IX	5 hrs. 20 min.	More strongly positive

In this experiment bile pigment appeared in the plasma four hours after the removal of the liver and other abdominal viscera from a dog in which the circulation was maintained through the entire musculo-skeletal system after hepatectomy. The pigment increased in amount up to the death of the animal an hour and a half later.

Expt. II. Dog 2. Female. Weight 11 pounds.

January 18, 1924: Morphia and ether anesthesia. Vena cava ligated between right ovarian vein and renal veins. Left ovarian vein ligated at entrance to renal vein. Six hours later the animal uses its hind legs stiffly and gingerly.

January 19, 1924: Uses hind legs normally. Runs about freely. No oedema.

February 5, 1924: Weight 12 pounds. Condition excellent. Veins of abdominal wall are moderately enlarged.

Morphia and ether anesthesia. Liver and other abdominal viscera removed exactly as described in Expt. I. Aorta not tied. Abdomen filled with warm packs and closed. Operation finished 11:30 a.m. Animal in excellent condition. Femoral arteries pulse well. 11:40 a.m., 8 cc. blood taken from jugular vein for test (Spec. I). 11:50 a.m., 35 cc. 10 per cent glucose intravenously. 12:30 p.m., 8 cc. blood taken (Spec. II). 1:30 p.m., 8 cc. blood taken (Spec. III). Condition excellent. 1:50 p.m., 30 cc. 10 per cent glucose. 2:30 p.m., 8 cc. blood taken (Spec. IV). 3:45 p.m., 8 cc. blood taken (Spec. V). Excellent condition. Pulse in femoral arteries good. Given 30 cc. 10 per cent glucose intravenously. 4:30 p.m., 8 cc. blood taken (Spec. VI). Moves feet normally. Condition excellent. 5:45 p.m., 8 cc. blood taken (Spec. VII). 15 cc. 20 per cent glucose intravenously. 7:50 p.m., condition excellent; 8 cc. blood taken (Spec. VIII). Immediately afterward 15 cc. 20 per cent glucose intravenously. 10:50 p.m., condition excellent. Femoral pulse good on both sides. 8 cc. blood taken (Spec. IX), followed by 15 cc. 20 per cent glucose intravenously. 12:50 a.m., condition continues good. 8 cc. blood taken (Spec. X) followed by 15 cc. 20 per cent glucose. 1:45 a.m., condition still excellent. 8 cc. blood taken (Spec. XI) after which the animal was killed.

Bilirubin tests on specimens of blood

NUMBER OF SPECIMEN	TIME AFTER REMOVAL OF LIVER	VAN DEN BERGH TEST (INDIRECT)
I	10 min.	Negative
II	1 hr.	Negative
III	2 hrs.	Negative
IV	3 hrs.	Negative
V	4 hrs. 15 min.	Negative
VI	5 hrs.	Negative
VII	6 hrs. 15 min.	Faintly positive
VIII	8 hrs. 20 min.	More faintly positive; too faint to read in colorimeter
IX	11 hrs. 20 min.	More faintly positive than previous specimen
X	13 hrs. 20 min.	Negative. Absolutely colorless.
XI	14 hrs. 15 min.	Negative. Absolutely colorless

Autopsy: Performed at once. The subcutaneous and epicardial fat is pale yellow in color and an alcoholic extract of this fat gives a faintly positive test for bile pigment. The heart and lungs are normal. The liver has been completely removed.

The peritoneal cavity is moist with several cubic centimetres of blood-tinged serum. All ligatures are intact. The old vena cava ligature completely occludes the vessel. The aorta and the iliac and femoral arteries are patent. A cannula is inserted into a small vein just above the left foot and a concentrated solution of Berlin blue is injected with very moderate pressure. The blue solution promptly enters the right side of the heart, showing that there are ample venous return channels from the hind limbs to the heart in spite of the ligated vena cava.

In this experiment bile pigment made its appearance in the plasma six and a quarter hours after the removal of the liver and all abdominal viscera from a dog in which the circulation was maintained through the entire musculo-skeletal system. But instead of increasing in amount up to the end of the experiment as in Experiment I, the specimen taken two hours after the first appearance of the pigment showed a decided *decrease* in the amount of bilirubin. Eleven hours after hepatectomy the bile pigment content of the serum was barely visible with the sensitive van den Bergh test and at the end of thirteen and a quarter hours after removal of the liver the serum was absolutely free from any detectable trace of bilirubin and remained so to the end of the experiment an hour later. The alcohol extract of these last two specimens was as clear as water and remained so after the addition of the diazo reagent. There is the possibility that bile pigment ceased to be formed at the point where the serum began to show a diminution in bilirubin content and that the small amount present then in the plasma passed into the fat tissue, which gave a faintly positive reaction for the pigment at the end of the experiment; or there is the possibility that the pigment continued to be formed, but that it passed into the fat tissue as rapidly as it appeared in the plasma. Many observations on the relation between the pigmentation of the plasma and of the fat of dogs at different stages of haemolytic and obstructive jaundice support the belief that the former possibility is the more probable one. I have never observed a tissue jaundice in the absence of a plasma jaundice at any time during the active liberation of bilirubin into the blood stream. It may be concluded here that, in this dog, bile pigment was formed slowly and in small amount following hepatectomy and then, for some reason, ceased to be formed entirely during the remaining eight hours of the experiment.

Expt. III. Dog. 7. Female. Weight 17½ pounds.

January 26, 1924: Morphia and ether anesthesia. Vena cava ligated between right ovarian vein and renal veins. Left ovarian vein ligated at its entrance to renal vein. Six hours later the animal uses its hind legs rather stiffly but can be made to run about with little difficulty.

January 27, 1924: Uses hind legs normally. Runs about.

February 14, 1924: Weight 18 pounds. Condition excellent. Veins of abdominal wall markedly enlarged.

Morphia and ether anesthesia. Liver and all other abdominal viscera removed exactly as described in Expt. I. Abdomen packed with warm towels and closed. Operation finished at 11 a.m. Animal in good condition. Femoral arteries pulsate well. 11:10 a.m., 8 cc. blood taken from jugular vein (Specimen I). 11:30 a.m., 40 cc. 10 per cent glucose intravenously. 11:45, 8 cc. blood taken (Spec. II). 12:45 p.m., restless and struggling. Wide awake. Condition excellent. Given ½ grain mor-

phia. 1:30 p.m., 8 cc. blood taken (Spec. III) followed by 20 cc. 20 per cent glucose intravenously. Whimpering. 2:55 p.m., struggling violently, using hind legs, whining. Given ½ grain morphia. Femoral pulse strong. 3 p.m., 8 cc. blood taken (Spec. IV). 3:30 p.m., 20 cc. 20 per cent glucose intravenously. Condition excellent. Wide awake and whining. Moves hind legs vigorously when toes are pinched. 4 p.m., 8 cc. blood taken (Spec. V). 5 p.m., 8 cc. blood taken (Spec. VI). Condition as before. Femoral pulse good on both sides. 5:20 p.m., 40 cc. 10 per cent glucose intravenously. 6:15 p.m., condition poor. 8 cc. blood taken (Spec. VII). Animal died at 6:25 p.m.

Autopsy: Performed at once. Subcutaneous and epicardial fat normally whitish in color. Alcohol extract of 10 grams of fat is negative for bile pigment. The heart and lungs are normal. The liver has been completely removed. There are a few small clots of blood in the peritoneal cavity. All ligatures are intact. The old vena cava ligature completely occludes the vessel. The aorta and the iliac and femoral arteries are patent. A cannula is inserted into a small vein above the foot and a concentrated solution of Berlin blue injected under very moderate pressure. The blue solution appears promptly in the right side of the heart, showing that there are patent venous return channels from the hind limbs in spite of the ligature on the vena cava.

Bilirubin tests on blood taken during the experiment

NUMBER OF SPECIMEN	TIME AFTER REMOVAL OF LIVER	VAN DEN BERGH TEST (INDIRECT)
I	10 min.	Negative
II	45 min.	Negative
III	2 hrs. 30 min.	Negative
IV	4 hrs.	Negative
V	5 hrs.	Negative
VI	6 hrs.	Negative
VII	7 hrs. 15 min.	Negative

In this experiment not a trace of bile pigment appeared in the plasma during seven and a quarter hours after the removal of the liver and all other abodminal viscera from a dog in which the circulation was maintained through the entire musculo-skeletal system.

An experiment was next carried out in which, after the establishment of a collateral circulation for the hind parts by preliminary ligature of the vena cava, the liver was removed and haemoglobin was injected into the circulation, in order to determine whether the delivery of abundant free haemoglobin to such an animal would be followed by a more marked production of bile pigment.

Expt. IV. Dog 10. Female. Weight 13½ pounds.

January 31, 1924: Morphia and ether anesthesia. Vena cava ligated between right ovarian vein and renal veins. Left ovarian vein ligated at entrance to renal vein. Six hours later moves hind legs with difficulty.

February 1, 1924: Can run about easily with no undue fatigue of hind legs. No oedema whatever.

February 21, 1924: Weight 16½ pounds. Condition excellent. No very marked dilatation of superficial abdominal veins.

Morphia and ether anesthesia. Liver and all other abdominal viscera removed exactly as described in Expt. I. Aorta not tied. Abdomen packed with warm towels and closed. Operation finished at 11:10 a.m. Condition very good. 11:30 a.m., 8 cc. blood taken from jugular vein (Spec. I). 12 noon, 20 cc. 20 per cent glucose intravenously, followed by 20 cc. laked red cells. The washed packed red cells from 40 cc. of blood were laked in 60 cc. distilled water. (20 cc. of this solution were used.) 1 p.m., condition excellent; 8 cc. blood taken (Spec. II). 2 p.m., 20 cc. 20 per cent glucose intravenously after taking 8 cc. blood (Spec. III). 3 p.m., condition very good. 8 cc. blood taken (Spec. IV). 4 p.m., moves hind legs vigorously when they are pinched. 20 cc. 20 per cent glu-

cose intravenously. 5 p.m., condition excellent. Femoral pulses of good quality. 8 cc. blood taken (Spec. VI). 6 p.m., 8 cc. blood taken (Spec. VII). Condition good. Killed with ether.

Autopsy: Performed at once. The subcutaneous and epicardial fat is dirty yellow in color and an alcohol extract of this fat gives a positive test for bile pigment. The heart and lungs are normal. The liver has been totally removed. The peritoneal cavity is free from blood. All ligatures are intact. The old vena cava ligature completely occludes that vessel. The aorta and the iliac and femoral arteries are patent. A cannula is introduced into a small vein above the foot and a Berlin blue solution injected under moderate pressure appears promptly in the right side of the heart, demonstrating that there were ample venous return channels from the hind legs notwithstanding the ligature on the vena cava.

Bilirubin tests on specimens of blood

NUMBER OF SPECIMEN	TIME AFTER REMOVAL OF LIVER	VAN DEN BERGH TEST (INDIRECT)
I	20 min.	Negative
II	1 hr. 50 min.	Extremely faint positive
III	2 hrs. 50 min.	Definitely positive
IV	3 hrs. 50 min.	More strongly positive
V	4 hrs. 50 min.	Positive
VI	5 hrs. 50 min.	Positive

In this experiment the addition of haemoglobin to the circulating blood was followed by the appearance of bilirubin in the plasma in a time much shorter, and in amount much greater, than in any of the previous experiments without haemoglobin injection.

The above experiments confirm the conclusion of Mann, Bollman and Magath (9) that bile pigment can be formed in an animal from which the liver and all the other abdominal viscera have been removed, if the circulation be maintained through the entire musculo-skeletal system. The conclusion which I drew, in my earlier paper (4), from experiments in which the circulation was much more restricted, was too sweeping.

The present method of removing the liver without any preliminary restriction of its circulation excludes any objection that, in the experiments of Mann and his associates, an abnormal, compensatory, extrahepatic mechanism might have been induced to develop during their preliminary period of reduction of hepatic function. There undoubtedly exists, outside the liver, a mechanism which, if it is not continually active in bile pigment production under normal conditions, can certainly assume that function on immediate notice. Whether, normally, the total daily output of bile pigment is manufactured exclusively by this extrahepatic mechanism—whether the liver plays any rôle at all other than a purely excretory one in regard to the pigment—is a matter which has been settled neither by the experiments of Mann, Bollman and Magath nor by the above experiments. Mann and his co-workers feel that bile pigment is formed as rapidly without a liver as with one. Makino, who used the same method of liver extirpation, gives figures to show that, in the presence of an active circulation through the entire body, including the abdominal viscera as well as the complete musculo-skeletal system, only about one-fifth of the normal amount of bile pigment is formed in the absence of the liver; and this figure was increased to about one-

fourth of the normal amount if haemoglobin were injected intravenously in large amounts (6). The present experiments, in which all the other abdominal viscera were removed, together with the liver, show even a much smaller production of bile pigment. One cannot, of course, assume that the subject of such an operation as hepatectomy or evisceration will continue to perform any particular bodily function in full vigor, and it would, therefore, be unsound to ascribe to the liver itself the production of the normal amount minus the amount produced outside it under such circumstances. The following experiments were carried out in the effort to learn to what extent the liver plays a rôle in the production of bile pigment.

II. Experiments with restricted circulation and evisceration in the presence of the liver

It will be remembered that, when the circulation was restricted to the head and thorax in my previous experiments (4) (or to a "heart-lung circulation" preparation in the experiments of McNee and Prusik (8)), bile pigment was not formed in the absence of the liver in any case, although a large amount of hemoglobin was introduced into the circulation; but in experiments exactly similar in all respects, save for the fact that the musculo-skeletal structures below the diaphragm were left in the circulation, bile pigment has appeared in the plasma. It seems desirable, therefore, to see what would happen in animals with a restricted circulation, which were subjected to the operation of evisceration of all abdominal organs *except* the liver.

In these experiments the circulation was restricted to the tissues of the head and thorax except for the fact that the arterial blood supply to the liver was left intact. The cystic duct and the common bile-duct were ligated in order that any bile formed might not escape by those paths. Such experiments were intended to represent the addition of at least a part of whatever bilirubin-making potentialities the liver may possess, to the head-thorax circulation experiment. Since the pigment never appeared in the head-thorax circulation experiments when the liver was totally excluded (4), it was felt that if bilirubin appeared promptly in the plasma in the experiments, it would point to activity of the liver itself in bilirubin production. Evisceration was carried out purposely, in order to subject the animals to as severe an operation as had been performed in the pure head-thorax circulation experiments.

Expt. V. Dog 12. Male. Weight 15 pounds.

Morphia and ether anesthesia. Midline incision. Ligation of all branches of coeliac axis *except the hepatic artery*. Ligation of aorta below coeliac axis. Ligation of mesenteric arteries, of renal vessels and of vena cava below liver. Common duct and portal vein ligated and divided at hilum of liver. Ligation of oesophagus just below diaphragm. Removal of stomach, intestines, spleen, pancreas, kidneys and bladder. The peritoneal cavity is free from bleeding. *The hepatic artery pulsates strongly up to the entrance of its branches into the substance of the liver.* Abdomen packed with warm towels and closed, care being taken to prevent pressure of the towels upon the hepatic artery.

Operation completed at 11:15 a.m., 8 cc. blood taken (Spec. I); 15 cc. 20 per cent glucose intravenously immediately thereafter. Twelve noon, condition good, 8 cc. blood taken (Spec. II). 12:15 p.m., 8 cc. blood taken (Spec. III). 12:45 p.m. condition not very good; 8 cc. blood taken (Spec. IV). 1 p.m., 8 cc. blood taken (Spec. V). Animal gasping for breath. Died 1:23 p.m.

Autopsy: Performed at once. Peritoneal cavity free from bloody fluid. All ligatures intact. Hepatic artery opened from coeliac axis to liver and found to be patent. No areas suggesting necrosis are seen in liver. When liver is cut, all bile-ducts are found filled with light brown bile. Liver appears normal microscopically. Heart and lungs normal. Body fat is colorless and is not tested for bile pigment.

Bilirubin tests on blood taken during experiment

NUMBER OF SPECIMEN	TIME AFTER OPERATION	VAN DEN BERGH TEST (INDIRECT)
I	5 min.	Negative
II	45 min.	Negative
III	1 hr.	Very faintly positive
IV	1 hr. 30 min.	Definitely positive
V	1 hr. 45 min.	More strongly positive

In this experiment bile pigment appeared in the plasma of a dog with a head-thorax circulation *plus* the arterial supply to the liver, one hour after establishment of the head-thorax circulation and removal of all abdominal organs except the liver. The bile pigment increased in amount up to the death of the animal one hour and forty-five minutes after the operation.

Expt. VI. Dog 14. Female. Weight 14 pounds.

Morphia and ether anesthesia. Operation for removal of all abdominal organs except liver carried out exactly as described in Expt. V, except that the strip of duodenum extending from the pylorus to a point just below the entrance of the bile duct is left within the body. This strip is washed free of contents and ligated at each end. The cystic duct is ligated and the gall-bladder removed. The common duct is cut between ligatures. Aorta tied just below coeliac axis. Vena cava ligated above renal veins. *Hepatic artery is left intact and pulsates strongly.* Abdomen packed with warm towels as before and closed. Operation finished 12 noon; 8 cc. blood taken from jugular vein (Spec. I). 15 cc. 20 per cent glucose intravenously. 1 p.m., 8 cc. blood taken (Spec. II). Condition excellent; restless; ½ grain morphia. 2 p.m., 8 cc. blood taken (Spec. III). Condition good. 2:25 p.m., heart has stopped. Spasmodic gasping. Thorax opened at once and blood withdrawn from heart (Spec. IV).

Autopsy: Performed at once. Heart and lungs are normal. There is a very slight amount (about 5 cc.) of blood-tinged fluid in the peritoneal cavity. All ligatures are intact. Hepatic artery is opened from coeliac axis to liver and is found patent. Intrahepatic bile ducts are filled with light brown bile. No areas suggesting necrosis are seen in liver, and microscopic sections of the organ appear normal. The body fat is colorless and does not give a test for bile pigment.

Bilirubin tests on blood taken during experiment

NUMBER OF SPECIMEN	TIME AFTER OPERATION	VAN DEN BERGH TEST (INDIRECT)
I	Immediately after	Negative
II	1 hr.	Negative
III	2 hrs.	Negative
IV	2 hrs. 25 min.	Faintly but definitely positive

In this experiment bile pigment appeared in the plasma of a dog with a head-thorax circulation *plus* the arterial supply to the liver, two hours and a half after establishment of the

head-thorax circulation and removal of all abdominal organs except the liver, the bile-ducts of which were obstructed.

In the next experiment hemoglobin was injected into the circulation of an animal after exactly similar operative procedures.

Expt. VII. Dog 15. Female. Weight 26 pounds.

Morphia and ether anesthesia. Operation for removal of all abdominal organs except the liver carried out exactly as described in Expt. V. Aorta tied below coeliac axis, and vena cava just below liver. *Hepatic artery left intact and pulsates strongly up to its entrance into liver.* Lymphatics at hilum are small and colorless. Abdomen packed with warm towels and closed. Operation finished 10:45 a.m. 11 a.m., 24 cc. laked red cells followed by 20 cc. 20 per cent glucose injected intravenously. (The washed, packed red cells from 40 cc. of blood were laked in 60 cc. distilled water and 24 cc. of this solution were used.) 8 cc. blood taken from jugular vein (Spec. I). 11:30 a.m., 8 cc. blood taken (Spec. II). Condition excellent. 12 noon, 8 cc. blood taken (Spec. III). 12:30 p.m., condition very good. Struggling. Given ½ grain morphia. 8 cc. blood taken (Spec. IV); immediately thereafter 17 cc. 20 per cent glucose given intravenously. 1 p.m., 8 cc. blood taken (Spec. V). 1:30 p.m., 8 cc. blood taken (Spec. VI). Condition very good. 2 p.m., 8 cc. blood taken (Spec. VII). 3 p.m., condition not very good; breathing is shallow and irregular, pulse very rapid. 8 cc. blood taken (Spec. VIII). Animal died with gasping respiration at 3:15 p.m.

Autopsy: Performed at once. The heart and lungs are normal. The fat over the thorax as well as the epicardial fat is yellowish in color and gives a positive test for bile pigment. All ligatures are intact. *The hepatic artery is patent.* The lymphatics draining the liver, which were colorless at the beginning of the experiment, are now greatly distended with a brownish-yellow lymph behind the ligature at the hilum which includes them. The bile-ducts in the substance of the liver are full of brown bile which smears the cut surface of the organ. The liver shows no gross evidence of necrosis and appears normal microscopically, except that the Kupffer cells contain a brown pigment. The peritoneal cavity is free from bloody fluid.

Bilirubin tests on blood taken during experiment

NUMBER OF SPECIMEN	TIME AFTER OPERATION	VAN DEN BERGH TEST (INDIRECT)
I	15 min.	Negative
II	45 min.	Negative
III	1 hr. 15 min.	Faintly but definitely positive
IV	1 hr. 45 min.	More strongly positive
V	2 hrs. 15 min.	More strongly positive
VI	2 hrs. 45 min.	Strongly positive
VII	3 hrs. 15 min.	Strongly positive
VIII	4 hrs. 15 min.	Strongly positive

In this experiment the addition of hemoglobin to the circulating blood of a dog with a head-thorax circulation *plus* the arterial circulation of the liver, was followed within one hour and fifteen minutes by the appearance of bile pigment in the plasma. The bilirubin increased in amount in the plasma up to the death of the animal four hours and a quarter after the operation. A definite tissue jaundice was present at the end of the experiment.

Expt. VIII. Dog 20. Female. Weight 19 pounds.

Morphia and ether anesthesia. Operative procedures exactly as in previous experiments to produce a head-thorax circulation *plus* the arterial circulation of the liver. Evisceration of remaining abdominal contents. Cystic duct and common bile-duct ligated. Abdomen packed with warm towels and closed. Operation finished 11:15 a.m. 11:30 a. m., given 15

cc. 20 per cent glucose intravenously, followed by 15 cc. laked red cells (20 cc. washed, packed red cells were laked in 65 cc. distilled water. 15 cc. of this solution were used). 8 cc. blood taken from jugular vein (Spec. I). 12:10 p.m., condition good. 8 cc. blood taken (Spec. II). 12:35 p.m., 8 cc. blood taken (Spec. III). 1 p.m., 8 cc. blood taken (Spec. IV). Condition good. 1:30 p.m., 8 cc. blood taken. Condition fair. 15 cc. 20 per cent glucose intravenously. 1:55 p.m., animal died with gasping respiration.

Autopsy: Performed at once. Subcutaneous and epicardial fat have a more yellowish tinge than normally, but no definite test for bile pigment can be obtained from it. No free fluid in abdominal cavity. All ligatures are intact. The hepatic artery is opened throughout its extrahepatic extent and is found patent. The intrahepatic bile-ducts are filled with brown bile. When the liver is cut the bile streams out of these ducts and smears the surface of the organ. No necroses are found either macroscopically or in the microscopic sections of the liver.

Bilirubin tests on blood taken during experiment

NUMBER OF SPECIMEN	TIME AFTER OPERATION	VAN DEN BERGH TEST (INDIRECT)
I	15 min.	Negative
II	55 min.	Negative
III	1 hr. 20 min.	Negative
IV	1 hr. 45 min.	Negative
V	2 hrs. 15 min.	Negative

In this experiment no bilirubin appeared in the plasma of a dog during two hours and fifteen minutes following the injection of hemoglobin into the circulation restricted to the head, thorax and liver.

DISCUSSION

The above experiments demonstrate that, if the circulation be maintained through the entire musculo-skeletal system after the complete removal of the liver and all the other abdominal organs from a dog, the plasma, which immediately after the operation is colorless and contains no detectable trace of bile pigment, may become yellow in color after some hours and give a positive test for bilirubin; that the bilirubin may increase gradually in amount in the plasma; and that a definite tissue jaundice may develop after the lapse of sufficient time. This confirms the important work of Mann, Bollman and Magath who were the first to demonstrate that the formation of bile pigment can proceed in an animal in which no blood is passing through the liver. The possibility of an extrahepatic formation of bile pigment has, for many years been the subject of much discussion but of little actual experimental study. Even the very recent literature contains numerous papers in which it is denied that the pigment can be formed outside the epithelial hepatic cell. As a matter of fact, there is no shred of proof that the epithelial liver cell has the power to manufacture bile pigment under any conditions. On the other hand, one can now reckon with certainty on the fact that the pigment may continue to be formed and to increase in the plasma to the point of tissue jaundice in an animal from which the liver has been completely removed.

The present method of removing the liver without having previously interfered with its blood supply excludes the objection that in the experiments of Mann, Bollman and Magath the formation of bile pigment after hepatectomy may have been

the result of the development of a compensatory mechanism on the part of extrahepatic tissues because of the prolonged reduction of the hepatic blood supply which Mann's method of hepatectomy necessitates. It is clear, therefore, that there exists outside the liver some ready mechanism which is able to form bile pigment independently of any activity of the liver cells, and it becomes entirely probable that this extrahepatic mechanism may be continually in operation under normal conditions. What is the nature of this bile-pigment-forming mechanism? It is possible, as has been repeatedly assumed (without, however, the least proof) that there exist extracellular enzymes capable of transforming hemoglobin into bile pigment and that the transformation can be effected within the circulating blood or tissue fluids. It is of immediate importance that a thorough search for such an enzyme be carried out. On the other hand, if the formation of bile pigment takes place within cells it seems most probable, from what we know of the distribution of the reticulo-endothelial phagocytes and of their specific activity in erythrophagocytosis, that these would be the cells concerned in the splitting of hemoglobin into bile pigment and an iron-containing residue. If the reticulo-endothelial cells are the active agents in bile pigment formation, the great richness of the liver in these cells would indicate at once its importance in this connection. Experiments are described above which indicate that bile pigment is formed more readily when blood passes through the liver than when it does not. The time required for the appearance of bile pigment in the plasma after ligation of the cystic duct and common bile-duct cannot, however, be thought to be an accurate index of the rapidity with which bile pigment is formed in any series of experiments. In different animals the bile channels are able to accommodate different amounts of bile before a retention of pigment in the plasma begins; and variations in the excretory function of the liver cells following the operation undoubtedly occur, as is shown by the abnormal excretion of bile during the first day after the establishment of a bile fistula. The above experiments of this nature are offered, therefore, chiefly for comparison with somewhat similar experiments of Mann, Bollman and Magath, who state that bile pigment appears in the plasma as soon after hepatectomy as after ligation of the bile-ducts. This was not the case in the present experiments, and even if it had been, it would not mean that the same amount of bile pigment was formed without a liver as with one, for one cannot neglect the amount of pigment which is formed and thrown into the bile channels before plasma jaundice appears after duct ligation. Indeed, Mann, Bollman and Magath state in another place that less bile pigment is formed without a liver than in the presence of this organ.

Statements that ordinary capillary endothelium or other types of fixed tissue cells are able to form bile pigment rest upon no proof whatever and, indeed, upon no direct evidence of any sort. Although there is a certain amount of quite suggestive

evidence in favor of the reticulo-endothelial cells as the site of origin of the pigment, this idea is by no means yet established as a fact. The writer has attempted to observe directly the changes which hemoglobin undergoes after being phagocytized by such cells in tissue cultures, but although bile pigment appeared regularly within the cells under such circumstances, and it was certain that it appeared in crystal form first within cells, we have not yet been able satisfactorily to exclude the possibility of the extra-cellular origin of the pigment and its phagocytosis in fluid form from the culture medium. We are unable to speak at present with any certainty about the mechanism by which bile pigment is formed in an hepatectomized animal, and the normal site of origin of bile pigment still remains a problem.

SUMMARY

1. Bile pigment may continue to be formed to the point of tissue jaundice in dogs from which the liver and all other abdominal viscera have been removed if an active circulation be maintained throughout the entire musculo-skeletal system. The amount of bile pigment formed after hepatectomy may be increased by the intravenous injection of hemoglobin.

2. Bile pigment may continue to be formed after the liver and all other abdominal organs have been removed from a dog by a method which involves no preliminary interference with the hepatic blood supply. This excludes the objection that the formation of bile pigment in the hepatectomized dogs of Mann, Bollman and Magath might have been the result of the development of a pathological compensatory extrahepatic mechanism. There exists an as yet unidentified extrahepatic mechanism which, if it is not continually active under normal conditions, is undoubtedly able to form bile pigment on immediate notice in the absence of the liver.

3. In contrast with the failure of bile pigment to appear in the plasma or tissues in experiments in which the circulation was restricted to the head and thorax, the pigment may appear in a time much shorter than the duration of these experiments if the arterial blood supply to the liver be added to the "head-thorax circulation" after obstruction of the common and cystic ducts.

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Studies on the Biology of the Streptococcus erysipelatis

I. Agglutination and Agglutinin Absorption with the Streptococcus erysipelatis[1, 2]

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INTRODUCTION

IT IS generally admitted that Streptococcus hemolyticus is the cause of erysipelas, although Jordan (1), Jochmann (2), and Reiche (3), have described cases of erysipelas due to staphylococci, and Neufeld (4) has concluded that the pneumococcus may produce the disease. Recent investigations have shown that Streptococcus hemolyticus is constantly associated with the skin lesions in erysipelas and quite often this organism has been recovered from the circulating blood in severe or fatal cases (5). Achard (6), however, notes the absence of Streptococcus hemolyticus in the desquamations of erysipelas. It has been found that the streptococcus plays a rôle in the production of erysipelas as shown by the accidental occurrence of erysipelatous lesions in human beings following the inoculations with Streptococcus hemolyticus for therapeutic purposes (7).

The work of Meierowitsch (8) in 1887 on the immunity produced by Streptococcus hemolyticus, followed a few years later by the studies of Roger (9), Grammakowsky (10), Denys and Leclef (11) and a number of other investigators, together with the recent investigations of Gay and Rhodes (12), have conclusively demonstrated that erysipelas can be produced experimentally by intradermal inoculation of Streptococcus hemolyticus and that the lesions produced in the skin of rabbits are not unlike those observed in human cases of erysipelas. Since then an abundant literature has accumulated dealing with the experimental production of erysipelas by the streptococcus. This subject has been thoroughly reviewed and summarized by Gay (13).

Up to the present time, however, the specificity of the particular variety of Streptococcus hemolyticus which is associated with erysipelas has not been established convincingly either by cultural or morphological studies. The following investigations were made, therefore, to ascertain whether a biological relationship existed be-

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[2] Received for publication February 19, 1925.

tween the individual strains of Streptococcus hemolyticus isolated from erysipelatous lesions. A variety of microörganisms have clearly been distinguished from one another by such immune reactions as specific agglutination and agglutinin absorption. These methods of biological classification of bacteria have been found most serviceable to differentiate varieties in the pneumococcus, typhoid, and Streptococcus scarlatinae groups, together with a number of other microörganisms. Many technical difficulties encountered in obtaining a uniform growth of streptococci and of reducing the tendency of all types of streptococcus to undergo spontaneous granulation have been recently overcome; and, consequently, in order to investigate a possible specific serological relationship between the streptococci isolated from erysipelatous lesions of erysipelas patients, we have resorted to the use of agglutination reactions and the determination of agglutinin absorption in the strains studied.

MATERIAL AND METHODS

The strains of Streptococcus hemolyticus employed in this series of investigations were isolated from typical cases of erysipelas occurring in New York, Boston, Washington, and Baltimore. Up to the present time thirty-four strains of erysipelatous origin have been studied. Nine of the strains were obtained from the serous fluid aspirated from subcutaneous abscesses in the lesions on the face and arms. Seventeen of the strains included in this series were obtained by the following technique: Physiological saline solution (0.5 to 1 cc.) was injected intradermally and subdermally along the edge of the extending erysipelatous lesion. This produced a small bleb from which fluid was immediately aspirated, without withdrawal of the needle. The fluid withdrawn was at once mixed with melted blood-agar, which was poured into Petri dishes according to the method described by Brown (14). Streptococcus hemolyticus was present in 91 per cent of the plates poured with the fluid aspirated from the margin of the advancing erysipelatous lesion. Similar cultures made from the fluid withdrawn from the less inflamed, central portion of the erysipelatous lesion yielded streptococci in only 46 per cent of the plates. Swab cultures made from the unabraded skin yielded staphylococci, while from the broken and suppurating lesions of the skin a mixture of streptococci and staphylococci was obtained in culture. The contents of the blebs associated with erysipelas gave sterile cultures in five out of eight individuals. The remaining patients showed each a small number of Streptococcus hemolyticus present in pure culture in the bleb fluid. It was found that the streptococci isolated from these typical cases of erysipelas did not differ morphologically or culturally from members of the large group of pyogenic streptococci. It was at once obvious, therefore, that we would be unable to classify the streptococci associated with erysipelas by any available cultural or morphological methods.

PATHOGENICITY

The virulence of the erysipelatous strains of Streptococcus hemolyticus proved to be different for mice,

guinea-pigs, dogs, and rabbits. The dog and rabbit were most susceptible. In rabbits the lethal dose of an 18-hour broth culture of strain "B_3" was 4 cc. per kilogram of body-weight. Death usually occurred in from 4 to 72 hours after inoculation. The most virulent was strain "C_4," which had been intensified in pathogenicity by five intracutaneous passages through rabbits. The lethal dose of an 18-hour broth culture of this strain was 2 cc. per kilogram of body-weight. Death occurred within from 4 to 16 hours after the inoculation. Frequent intracutaneous passage through rabbits and dogs has been resorted to, in order to preserve, in so far as possible, the original virulence of the strains studied.

Since it has been found that the serum of normal rabbits may agglutinate hemolytic streptococci, the serum of each animal that was to be used for these experiments was tested before inoculation for its power to agglutinate four erysipelatous and four non-erysipelatous strains of Streptococcus hemolyticus. Of the stock rabbits 23 per cent possessed natural agglutinins for these streptococci and these animals were consequently discarded.

METHODS OF INOCULATION

Three methods of inoculation were employed for the production of immune serum: (A) intravenous, (B) intracutaneous, and (C) intracutaneous in previously injected agar.

A. Intravenous inoculation. The method described by Stevens and Dochez (16) of intravenous inoculation was employed in some of these experiments. The first two series of inoculations were made with amounts of heat-killed 18-hour broth cultures of streptococci, varying from 0.5 to 4 cc. Each series consisted of 4 inoculations on consecutive days, an interval of 4 days being allowed to elapse between each series. The third and fourth series consisted of similar inoculations with living 18-hour broth cultures of hemolytic streptococci. If the animal lost weight during the process of immunization, the dosage of streptococci was decreased and the process slightly prolonged. The animals were anaesthetized and bled from the heart about the tenth day after the last inoculation, since it was found, by daily agglutination curves, that the highest agglutinin titer occurred about this time. The average titer for the strains of streptococci used in immunization by this method was found to be 1 to 1,280. Thirty per cent of the animals employed died during the third series of inoculations.

B. Intracutaneous inoculation. The hind legs of rabbits employed in this series were first depilated. An initial dose of 0.1 cc. of an 18-hour broth culture of living streptococci was injected intracutaneously in the muscles of the thigh. About the twelfth day after the initial inoculation, a second dose of 0.3 cc. of an 18-hour broth culture of living streptococci was injected intracutaneously on the other side of the body. An area previously inoculated with streptococci was avoided in subsequent re-inoculations. For the next six weeks, larger dosages of streptococci were injected every tenth day, until a single dose of 5 cc. was injected intracutaneously. Ten days after the last inoculation the animals were anaesthetized and bled from the heart, and the agglutinin titer was immediately determined. The average titer for the strains of streptococci used for immunization by this method

was found to be 1 to 2,560. Of the animals employed in this series 22 per cent died during the fourth to the sixth week in the course of immunization.

C. Intracutaneous inoculation in previously injected agar. The method of injecting agar intracutaneously previous to the inoculation with live streptococci for the production of immune bodies, was first described and made use of by Dochez (15). The hind legs of rabbits employed in this series were first depilated. Into the muscles of the thigh were injected 5 cc. of a 2 per cent beef infusion agar, the reaction of which had been previously adjusted to pH 7.4 and the temperature of which had been reduced to about 40°C. before injection. Following the injection of the agar, a Luer tuberculin syringe, containing 0.1 cc. of an 18-hour broth culture of live streptococci, was attached to the needle; and when the agar had hardened, the streptococcic emulsion was injected into it. It was found that about 70 per cent of the rabbits inoculated with strain "C_4" by this method died on the third and fourth day after the injection. This high mortality was greatly reduced when simultaneously with the injection of live streptococci into the agar the rabbits were inoculated intravenously with 1 to 2 cc. of heat-killed streptococci. A second injection of 0.5 cc. of agar, into which was inoculated 0.3 cc. of live streptococci, was given about twelve days after the first inoculation, usually on the other side of the body. For the next six weeks, larger dosages were injected, without the agar, until a single dose of 5 cc. of live streptococci was injected intracutaneously. Ten days after the last inoculation, the animals were anesthetized and bled from the heart. The average agglutinin titer of the serum against the strains of streptococci employed in this method of immunization was 1 to 5,120. Of the animals inoculated in this series 26 per cent died during the fourth and fifth week.

Inactivation of the immune sera at 56°C. for 30 minutes was found to accelerate the deterioration of the agglutinins in the anti-erysipelas sera. Freshly prepared sera, therefore, gave the sharpest agglutination reactions and the most definite results in the experiments upon absorption of agglutinins, in tests with both homologous and heterologous strains of Streptococcus hemolyticus.

SOURCE OF STRAINS

The thirty-four strains of erysipelatous Streptococcus hemolyticus represented in Table I were isolated during the summer and fall of 1924. These strains were obtained from typical cases of erysipelas by intradermal, subcutaneous, and bleb cultures, as well as from the suppurating skin lesions, as indicated in Table I.[3]

[3] The author is greatly indebted to Dr. T. M. Rivers, of the Rockefeller Institute for Medical Research, New York, for strains "A_1" and "B_2"; to Dr. Hans Zinsser, of Harvard Medical School, Boston, for strain "C_{34}"; to Mr. Milo R. White, of the Sydenham Hospital, Baltimore, for strains "C_{15-16}"; "C_{18-22}", and "C_{19-21}", to Dr. T. B. Futcher, Baltimore, for strain "M_1", to Dr. S. J. Crowe, of The Johns Hopkins Surgical Clinic, for strains "N_{1-5}", to Dr. A. R. Dochez, of the Presbyterian Hospital, New York, for strain "SF_{135}", and to Dr. John M. T. Finney, for permission to culture patients in The Johns Hopkins Surgical Clinic and the Union Memorial Hospital, Baltimore.

TABLE I

Source of strains of Streptococcus hemolyticus studied

STRAIN NUMBER	SOURCE	CLINICAL DIAGNOSIS	HEMOLYSIS	FERMENTATION REACTIONS			
				Lactose	Salicin	Mannite	Inulin
A_1, C_1, C_3, C_3, C_6, C_{11}, C_{12}, C_{16}, C_{17}, C_{18}, C_{24}, C_{26}, C_{27}, C_{30}, C_{32}	Skin (I.D.)	Erysipelas	++++	+	+	−	−
C_7	Skin (I.D.)	Erysipelas	++++	+	+	+	−
C_{10}, C_{19}, C_{20}, C_{21}, C_{25}, C_{28}	Skin (bleb)		++++	+	+	−	−
C_{22}	Skin (bleb)	Erysipelas	++++	+	+	+	−
B_2, C_4, C_5, C_8, C_9, C_{13}, C_{14}, C_{15}, C_{31}, C_{34}	Wound	Erysipelas	++++	+	+	−	−
C_{23}, C_{29}, C_{33}	Abscess	Erysipelas	++++	+	+	−	−
D_1	Skin (I.D.)	Cellulitis	++++	+	+	−	−
D_2	Wound	Cellulitis	++++	+	+	−	−
D_3, D_4, D_5	Skin (I.D.)	Dermatitis	++++	+	+	−	−
E_1, E_2, E_3, E_4	Pus	Empyema	++++	+	+	−	−
M_1, M_2, M_3, M_4	Pus	Mastoiditis	++++	+	+	−	−
N_2, N_3, N_4, N_6, N_7, N_8, N_9, N_{11}, N_{13}, N_{15}, N_{14}, N_{18}	Nose	Nasal sinusitis, acute	++++	+	+	−	−
N_5	Nose	Nasal sinusitis, chronic	++++	+	+	+	−
N_{16}, N_{17}	Nose	Nasal sinusitis, subacute	++++	+	+	−	−
P_1, P_2, P_3, P_4, P_5, P_6	Pus	Peritonitis	++++	+	+	−	−
Ob_1, Ob_2, Ob_3	Pus	Puerperal sepsis	++++	+	+	−	−
SF_{136}, SF_{224}, SF_{247}	Throat	Scarlet fever	++++	+	+	−	−
T_{21}, T_{22}	Throat	Tonsillitis	++++	+	+	−	−
W_7, W_{19}	Throat	Surgical wound	++++	+	+	−	−

I.D. indicates intradermal culture.

The forty-five strains of non-erysipelatous Streptococcus hemolyticus were isolated from a large variety of pyogenic infections and the source of these strains is indicated in Table I.

RESULTS OF AGGLUTINATION

Of the 34 strains of erysipelatous Streptococcus hemolyticus isolated from typical cases of erysipelas, seven were made use of for the production of immune erysipelas sera. Each immune serum bears the name and number of the strain of streptococcus employed in the preparation of the serum, and these are as follows: "A_1," "B_3," "C_1," "C_2," "C_4," "C_5," and "C_7." It was found by cross-agglutination of the 34 strains of erysipelatous streptococci against each of the seven immune erysipelas sera, that 31 strains, or 91.2 per cent of the streptococci from cases of erysipelas, were agglutinated by the serum from rabbits immunized against erysipelatous strains (Table II).

In order to investigate whether a possible serological relationship existed between the hemolytic streptococci isolated from erysipelatous lesions of cases with erysipelas and the hemolytic streptococci isolated from scarlet fever patients, two immune scarlatinal sera were employed in a series of agglutination reactions against the 34 strains of erysipelatous hemolytic streptococci. Anti-scarlatinal Serum "SF_1" was obtained through the kindness of Doctor A. R. Dochez, and serum "SF_2" was prepared in our own laboratory by Dochez' method for the production of anti-scarlatinal serum (15). No agglutination of the erysipelatous strains of Streptococcus hemolyticus occurred with the two immune scarlatinal sera. Further, it was found that of 23 strains of hemolytic streptococci isolated from the throat secretions of patients with scarlet fever, no agglutination occurred when these strains were employed against the immune erysipelas sera.

Forty-five strains of miscellaneous Streptococcus hemolyticus isolated from pyogenic infections other than erysipelas were employed in cross-agglutination against the seven immune erysipelas sera, in order to demonstrate any possible immunological relationships among the large group of pyogenic streptococci. The sources from which these streptococci were isolated included cellulitis, dermatitis, empyema, mastoiditis, meningitis, nasal sinusitis, peritonitis, puerperal sepsis, scarlet fever, tonsillitis and surgical wounds. It was found that nine strains among these, or 20 per cent, were agglutinated by seven immune erysipelas sera. The agglutinable non-erysipelatous strains of streptococci were obtained from the nasal secretions of individuals with chronic, subacute and acute sinusitis, and one strain isolated from the meninges of a case of meningitis following mastoiditis (Table III).

AGGLUTININ ABSORPTION

It was necessary, as was first pointed out by Stevens and Dochez (16) in their studies on the Streptococcus scarlatinae, to make certain combinations of the immune erysipelatous sera, and the erysipelatous strains, in order to obtain sharp and convincing reactions of the absorption of agglutinins. If the serum was freshly prepared, the erysipelatous strain would

TABLE II

Cross-agglutination of strains of Streptococcus hemolyticus isolated from skin lesions of clinical cases of erysipelas

STRAINS OF STREPTOCOCCI	IMMUNE SERA										
	A_1	B_2	C_1	C_2	C_4	C_6	C_7	D_1	SF_1	SF_2	T_1
A_1	++++	++++	+++	++	+++	+++	++	0	0	0	0
B_2	++++	++++	++	++	+++	±	++	0	0	0	0
C_1	+++	+++	++++	±	+++	±	+++	0	0	0	0
C_2	++	++	++	++++	+++	++	++	0	0	0	0
C_3	+++	+++	±	±	+	++	0	0	0	0	0
C_4	+++	+++	++	+++	++++	+++	++	0	0	0	0
C_5	++	++	++	++	+++	++++	+++	0	0	0	0
C_6	0	0	0	0	0	0	0	0	0	0	+++
C_7	++	+++	+++	0	++	±	++++	0	–	0	0
C_8	+	+	0	0	+++	++	+++	0	–	0	0 –
C_9	+++	+++	++	++	+++	+	+	–	0	–	–
C_{10}	++++	++++	–	–	+++	–	++	–	0	–	–
C_{11}	0	0	0	0	0	0	0	+++	0	0	0 –
C_{12}	–	+++	+++	++	++	–	–	0	–	0	0 –
C_{13}	–	++	–	+++	+++	–	–	0	–	0	– 0
C_{14}	–	++++	–	–	++++	–	++	0	–	0	0 –
C_{15}	–	++	–	++	+++	–	0	0	–	0	0 –
C_{16}	++	++	+++	++	++++	–	++	0	–	0	–
C_{17}	++++	++++	++++	++	++	+++	+	0	–	0	0 –
C_{18}	–	–	–	+++	++++	–	++	0	–	0	– 0
C_{19}	+++	+++	++	++	++++	–	–	0	0	–	0 –
C_{20}	++	–	+++	–	+++	–	++	–	–	–	0 –
C_{21}	0	0	++++	++	±	±	++	–	–	0	– –
C_{22}	++++	++++	+++	++	++++	++	++	–	–	0	– –
C_{23}	0	0	+++	++	++++	–	–	0	–	0	0 –
C_{24}	–	–	–	+++	++++	++	++	–	–	0	– –
C_{25}	–	–	–	++	++++	–	+++	–	–	0	– –
C_{26}	–	–	–	++	+	++++	++	0	–	0	0

C_{27}	+++	+++	++	0	+++	0	+	−	−	0	−
C_{28}	++	++	+	+	++++	++	++	−	0	−	0
C_{29}	−	+++	−	−	++++	−	+++	0	−	0	−
C_{30}	−	++	−	0	0	+++	+++	0	−	0	−
C_{31}	0	0	0	0	0	0	0	0	−	0	0
C_{32}	+++	++++	−	−	+++	−	++	0	−	0	−
C_{33}	+	++	++	+	0	++	+	−	−	0	−
C_{34}	−	+++	++	++++	+++	+	0	−	0	0	−
D_1	0	0	0	0	0	0	0	++++	0	0	0
SF_1	0	0	0	0	0	0	−	0	++++	+++	0
SF_2	0	−	−	0	0	−	−	0	+++	++++	0
T_1	0	−	−	0	0	0	0	0	0	0	++++

++++ indicates complete agglutination of 1 to 1,280 or higher, +++ of 1 to 640, ++ of 1 to 320, + of 1 to 160, ± complete or incomplete agglutination in the lower dilutions only. The letter 0 signifies negative agglutination, and the minus sign indicates that no test was made.

TABLE III

Cross-agglutination of strains of Streptococcus hemolyticus isolated from non-erysipelatous lesions

STRAINS OF STREPTOCOCCI	IMMUNE ERYSIPELAS SERA							STRAIN NUMBER
	A_1	B_2	C_1	C_2	C_4	C_6	C_7	
Cellulitis	0	0	0	0	0	0	0	D_1
	0	0	0	0	0	0	0	D_2
Dermatitis	0	0	0	0	0	0	0	D_3
	0	0	−	−	0	−	0	D_4
	−	0	0	−	0	−	0	D_5
Empyema	0	0	0	0	0	0	0	E_1
	−	0	−	−	0	−	0	E_2
	−	0	−	−	0	0	0	E_3
	0	0	0	0	0	0	0	E_4
Mastoiditis	+++	+++	0	++	++++	+	+++	M_1
	0	0	0	0	0	0	0	M_2
	0	0	−	−	0	−	0	M_3
	0	0	±	0	0	0	±	M_4
Nasal sinusitis	0	0	0	0	0	0	0	N_1
	+++	++++	++	0	++++	0	+++	N_2
	++	++	+	++	++++	0	++	N_3
	0	0	0	0	0	0	0	N_4
	0	0	0	0	0	0	0	N_5
	±	±	0	++++	+++	0	++++	N_6
	++++	++++	0	0	++	++	++++	N_7
	0	0	0	0	0	0	0	N_8
	0	−	0	−	0	0	0	N_9
	0	0	0	0	0	0	0	N_{10}
	+++	++++	++	+	++++	0	0	N_{11}
	0	0	0	0	0	0	0	N_{12}
	0	0	0	0	0	0	0	N_{13}
	++	+++	++	+	++++	0	++	N_{14}
	0	0	0	0	0	0	0	N_{15}
	+++	+++	0	++	++++	++	+++	N_{16}
	++	+++	+	±	0	0	++	N_{17}
	0	0	0	++++	0	+++	+++	N_{18}
Peritonitis	0	0	0	0	0	0	0	P_1
	0	0	−	0	−	0	0	P_2
	0	0	−	0	−	0	0	P_3
	0	0	0	0	0	0	−	P_6
Puerperal sepsis	0	0	0	0	0	0	0	O_1
	0	0	−	0	−	0	−	O_2
	0	0	0	−	0	−	0	O_3

Of 45 strains of non-erysipelas streptococci, 9 strains, or 20 per cent, agglutinated with the immune erysipelas sera.

TABLE III—*Continued*

STRAINS OF STREPTOCOCCI	IMMUNE ERYSIPELAS SERA							STRAIN NUMBER
	A_1	B_2	C_1	C_2	C_4	C_5	C_7	
Scarlet fever	0	0	0	0	0	0	0	SF_{126}
	0	0	0	0	0	0	0	SF_{234}
	0	0	0	0	0	0	0	SF_{257}
Tonsillitis	0	0	0	0	−	−	0	T_{23}
	0	0	−	−	0	0	0	T_{32}
Wounds	0	0	0	0	±	0	0	W_7
	−	0	−	0	0	0	0	W_{19}

TABLE IV

Agglutinin absorption. Serum A_1 absorbed with erysipelas strains A_1, B_2 and C_1, and non-erysipelas strains SF_1, D_1 and N_2

SERUM	TITER OF SERUM WITH STRAIN A_1					
	Dilutions					
	1:80	1:160	1:320	1:640	1:1280	1:2560
Control, normal serum	0	0	0	0	0	0
Immune serum A_1	+++	+++	+++	+++	++	+
Heated serum A_1	++	++	++	+	±	0
Serum A_1 absorbed with strain A_1	0	0	0	0	0	0
Serum A_1 absorbed with strain B_2	±	0	0	0	0	0
Serum A_1 absorbed with strain C_1	+	0	0	0	0	0
Serum A_1 absorbed with strain SF_1	++	+++	+++	++	+	0
Serum A_1 absorbed with strain D_1	++	+++	++	+	±	0
Serum A_1 absorbed with strain N_2	±	0	0	0	0	0

TABLE V

Agglutinin absorption. Serum B_2 absorbed with erysipelas strains B_2, C_2, C_3, C_4, C_5, and non-erysipelas strains D_1, N_2, SF_2

SERUM	TITER OF SERUM OF STRAIN B_2					
	Dilutions					
	1:80	1:160	1:320	1:640	1:1280	1:2560
Control, normal serum	0	0	0	0	0	0
Immune serum B_2	++++	++++	+++	+++	++	+
Heated serum B_2	+++	+++	+++	++	±	0
Serum B_2 absorbed with strain B_2	0	0	0	0	0	0
Serum [illegible] absorbed with strain C_2	+	±	0	0	0	0
Serum [illegible] absorbed with strain C_3	0	0	0	0	0	0
Serum B_2 absorbed with strain C_4	0	0	0	0	0	0
Serum B_2 absorbed with strain C_5	++	+++	+++	+++	++	±
Serum B_2 absorbed with strain D_1	++	+++	++	+	+	0
Serum B_2 absorbed with strain N_2	±	0	0	0	0	0
Serum SF_2 absorbed with strain SF_2	+++	+++	+++	++	+	0

absorb the agglutinins from the anti-erysipelatous serum completely, provided the agglutination reaction was complete within 30 minutes at 55°C. Throughout all the determinations of specific agglutinin absorption, the anti-erysipelatous serum was diluted 1 to 40, and was absorbed twice at 55°C. with streptococci obtained by centrifugalization of 200 cc. of an 18-hour broth culture. Before these streptococci were employed in the experiments to determine the specific agglutinin absorption, they were twice washed in sterile Locke's solution and re-centrifugalized. The absorption tests were always conducted in double series, under similar conditions and with identical technique. A few of the results of these experiments are shown in Tables IV and VI.

TABLE VI

Agglutinin absorption. Serum C_1 absorbed with erysipelas strains A_1, B_3, C_1, C_2, C_3, C_4, C_5, and non-erysipelas strains D_1, N_2

SERUM	TITER OF SERUM WITH STRAIN C_1					
	Dilutions					
	1:80	1:160	1:320	1:640	1:1281	1:2560
Control, normal serum	0	0	0	0	0	0
Immune serum C_1	+++	++++	++++	+++	++	±
Heated serum C_1	+++	+++	+++	++	±	0
Serum C_1 absorbed with strain C_1	0	0	0	0	0	0
Serum C_1 absorbed with strain A_1	±	0	0	0	0	0
Serum C_1 absorbed with strain B_3	±	0	0	0	0	0
Serum C_1 absorbed with strain C_2	+	0	0	0	0	0
Serum C_1 absorbed with strain C_3	±	0	0	0	0	0
Serum C_1 absorbed with strain C_4	+	0	0	0	0	0
Serum C_1 absorbed with strain C_5	+++	+++	+++	++	0	0
Serum C_1 absorbed with strain D_1	+++	++++	+++	+++	++	0
Serum C_1 absorbed with strain N_2	+	0	0	0	0	0

Every strain of hemolytic streptococci of erysipelatous origin was employed in the absorption reactions. The agglutinins in the immune serum produced by a given erysipelatous strain were completely absorbed by its homologous strain, as well as by a number of other strains of erysipelatous hemolytic streptococci, and the absorption of agglutinins from a number of immune erysipelatous sera was accomplished with each agglutinable erysipelatous strain.

These investigations demonstrate that at least 90 per cent of the strains of Streptococcus hemolyticus isolated from typical lesions of erysipelas fall immunologically, as determined by the agglutination reaction and the phenomenon of agglutinin absorption, into one group, and that this group of hemolytic streptococci can be differentiated, by these same immunological methods, from the group of hemolytic streptococci associated with scarlet fever.

SUMMARY

Intracutaneous injection of agar inoculated with live *Streptococcus hemolyticus* for the production of immune bodies was found to produce an

immune serum of a higher agglutinin titer than that obtained by the intravenous or subcutaneous inoculation of dead or live hemolytic streptococci.

Of 34 strains of erysipelatous hemolytic streptococci, seven were employed for the production of seven immune erysipelatous sera. Thirty-one strains of the thirty-four erysipelatous strains, or 91.2 per cent, agglutinated with each of seven immune erysipelatous sera.

Of 45 strains of hemolytic streptococci isolated from sources other than erysipelas, nine, or 20 per cent, were agglutinated by several immune erysipelas sera. Eight of the nine strains of Streptococcus hemolyticus, which were agglutinated by the serum from rabbits immunized against erysipelatous strains, were obtained from cases of nasal sinusitis.

Agglutinin absorption was accomplished under the conditions of the experiments with all the combinations of immune erysipelatous sera and strains of hemolytic streptococci of erysipelatous origin.

These experiments indicate that it is possible to differentiate by immunological methods a group of hemolytic streptococci causing erysipelas from the group of hemolytic streptococci responsible for scarlet fever, on the one hand, and on the other, from the large series of miscellaneous hemolytic streptococci producing a variety of pyogenic infections.

The author wishes to acknowledge his sincere appreciation to Doctor Harold L. Amoss for direction and suggestion of these investigations, and to Doctor J. H. Brown for invaluable criticism and suggestions during the course of these studies.

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Clinical and Pathological Studies on Chronic Nephrosis

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THE clinical and pathological picture of chronic nephrosis is shrouded by a certain amount of mystery. Although nephrosis presents certain clinical features in common with chronic nephritis and has been classed by some as merely a type of nephritis, most observers who have studied this condition feel that it has certain characteristics that stamp it as a definite disease entity.

Epstein (1), (2), (3), in this country, has called attention to the interesting disease picture of chronic nephrosis and has pointed out that its most striking features are the presence of hypercholesterinemia, a low metabolic rate and a great increase in the serum globulin as compared with the serum albumin.

The hypercholesterinemia is one of the striking features of the blood chemistry and may be present with a normal blood urea, creatinine, uric acid and chlorides. Müller (4) describes a patient with a blood cholesterol of 836 mgm. per 100 cc. Heusler's (5) patient showed a blood cholesterol of 225 mgm. and Rabinowitch and Childs (6) found 781 mgm. of cholesterol per 100 cc. in their case.

The low metabolic rate which these patients show has not been the subject of so much study as some of the other features of the disease. Epstein has emphasized his belief that this indicates a hypothyroid state and describes marked clinical improvement following the administration of thyroid extract. Kollert (7) has suggested that the edema, which is marked in many patients, is produced by the presence of split protein products in the tissues and that thyroid extract hastens the destruction of these products and clears up the edema.

In the normal serum the ratio of serum albumin to serum globulin is as 2:1. In nephrosis this ratio is disturbed and there may be a much greater percentage of globulin than of albumin. Epstein (3) describes a serum globulin of 89 per cent. Rabinowitch and Childs (6) found a globulin percentage of 66 in their patient, and Kollert (7) mentions a marked increase in globulin. These changes are accompanied by a heavy excretion of albumin in the urine.

All writers agree that the disease is comparatively rare. Rabinowitch and Childs saw only one patient on their service in three years, and Epstein mentions its infrequency. The reports of necropsies on these patients are also few in number.

Most articles describing this condition make no reference to necropsy findings and the total number that have been studied from the viewpoint of pathological anatomy is small.

Since we had an opportunity of studying the pathological anatomy of the kidneys in this unusual condition, the report of a case recently studied would seem worthy of record.

This patient was a man, aged thirty-four, who was admitted to the Bell Memorial Hospital on July 3, 1924, complaining of shortness of breath and swelling of the feet.

Family and personal history. Essentially negative.

Present illness. Began in November, 1923, with puffiness of the eyelids and swelling of the feet. This condition gradually increased until the patient's eyes, in the morning, were often almost closed by the edematous lids, while in the evening his feet were too much swollen to permit the wearing of shoes. At this time an examination of the urine showed albumin and he was placed on a milk diet and given magnesium sulphate every morning.

He also had his teeth examined and several were removed. In spite of treatment, however, the edema gradually grew worse and shortness of breath developed.

On physical examination the patient was somewhat dyspnoeic, showed a few moist râles at the bases of both lungs, marked edema of the back and of both legs and feet. The heart was not enlarged on physical examination and this finding was confirmed by the x-ray plate. The blood pressure was 140 systolic and 90 diastolic.

Examination of the urine showed a specific gravity of 1031, a heavy cloud of albumin and numerous hyaline casts. The first phenolsulphonephthalein test showed an excretion of 65 per cent in two hours; the creatinine test showed a total excretion of 10 mgm. for the first hour, 86 mgm. for the second hour and 54 mgm. for the third hour—an unusually good excretion.

An examination of the blood showed R. B. C. 4,100,000, W. B. C. 8500, Hg. 75 per cent. The Wassermann was negative. The blood urea on admission was 15 mgm. and the blood cholesterol 344 mgm. per 100 cc. The blood was quite milky in appearance, showing a marked lipemia. An examination of blood proteins showed the ratio of serum albumin to serum globulin to be as 1:2. The basal metabolic rate was −31 per cent. The temperature and pulse rate were normal.

The patient was placed on a diet of 250 grams of carbohydrate, 150 grams of protein and 50 grams of fat per day. For one week he was given daily 60 to 100 grains of calcium chloride by mouth. Ten days after admission he was started out on two grains of thyroid extract daily, and the dose was gradually increased to eight grains per day. The basal metabolic rate on July 31 was increased to −10 per cent.

None of these therapeutic measures had any constant effect on the course of his disease. Subjectively, he grew constantly worse, the edema persisted, although it varied from day to day, the urinary output continued small, although at times for a day there was an adequate excretion. The excretion of chlorides was continuously low with complete suppression on nine different days. The blood cholesterol fell somewhat, the blood chlorides continued to be low, as did the blood urea and blood sugar. The phenolsulphonephthalein test on July 3 was 22 per cent after two hours (Table 1).

The patient left the hospital on August 5, 1924, and went to his home. The edema became more and more marked, the skin of the leg broke and drained large quantitiess of serous fluid. The patient developed edema of the lungs and died one week after his discharge from the hospital.

A complete autopsy was not permitted, but consent was secured to the removal of both kidneys and a piece of the liver. Both kidneys were somewhat enlarged, the right measuring 13 x 7 by x 4 cm. and weighing 210 grams. The left was approximately the same size and weighed 220 grams. Both kidneys were somewhat pale and showed numerous small yellowish patches. The cortex was not thickened and the capsule stripped easily.

Microscopically, the kidney presented an

TABLE I

The blood urea, sugar, chlorides, cholesterol and creatinine are expressed in milligrams per 100 cc. The carbon dioxide tension in volumes per cent. The urinary nitrogen and chlorides are expressed in grams.

	BLOOD						URINE		
	Urea	Sugar	Chlorides	Cholesterol	Creatinine	CO_2	Amount	Total nitrogen	Total chlorides
July 4	14.0		450	344			500	8.9	0.6
July 5				366					
July 6									
July 7									
July 8							600	9.7	0.5
July 9							1200	10.0	1.68
July 10							1500	21.0	3.3
July 11		94				33.8	1100	8.5	0.3
July 12							1000	9.3	1.9
July 13							1400	14.5	2.1
July 14						24.2	500	6.1	0.7
July 15							1700	10.5	0.14
July 16							1000	7.8	0.4
July 17							800	10.0	0.16
July 18							650	6.7	0.45
July 19							500	5.3	0.3
July 20									
July 21			440			28.9			
July 22							1500	30.0	0.15
July 23							600	2.6	0.06
July 24	9.8					28.9			
July 25									
July 26							300	5.5	0.24
July 27							200	3.56	0.0
July 28							150	2.37	0.0
July 29							150	2.51	0.0
July 30	5.9	82	330	273	1.4		330	0.5	0.0
July 31							700	8.47	0.0
August 1							1000	10.4	0.0
August 2							1300	9.3	0.1
August 3							1600	11.2	0.0
August 4							500	7.2	0.0
August 5							500	7.2	0.0

interesting picture. Some of the glomeruli showed engorgement with red blood cells, and others showed swollen endothelial cells with numerous small clear vacuoles. The glomeruli showed a mild degree of acute glomerulitis, while chronic changes were mainly absent. The tubules showed varying degrees of degeneration with considerable dilatation and pressure atrophy (Fig. 1). Many of the cells in the tubules were swollen and contained large clear vacuoles. The interstitial tissue of the kidney showed numerous cellular patchy areas of fibrosis and also some areas where the stroma was

filled with large clear vacuoles, although the vacuoles were most numerous in the cells forming the loops of Henle.

When the kidneys were stained with Scharlach R and Sudan III the clear vacuoles seen in the hematoxylin and eosin preparations now took the fat stain. The character of the lipoid substance so abundant in the kidneys. Controls of adrenal and known fatty liver were used and Scharlach R, Neutral Red, Nile Blue Sulphate, Osmic acid, Ciaccio and Lorraine Smith-Dietrich stains were applied with the following results.

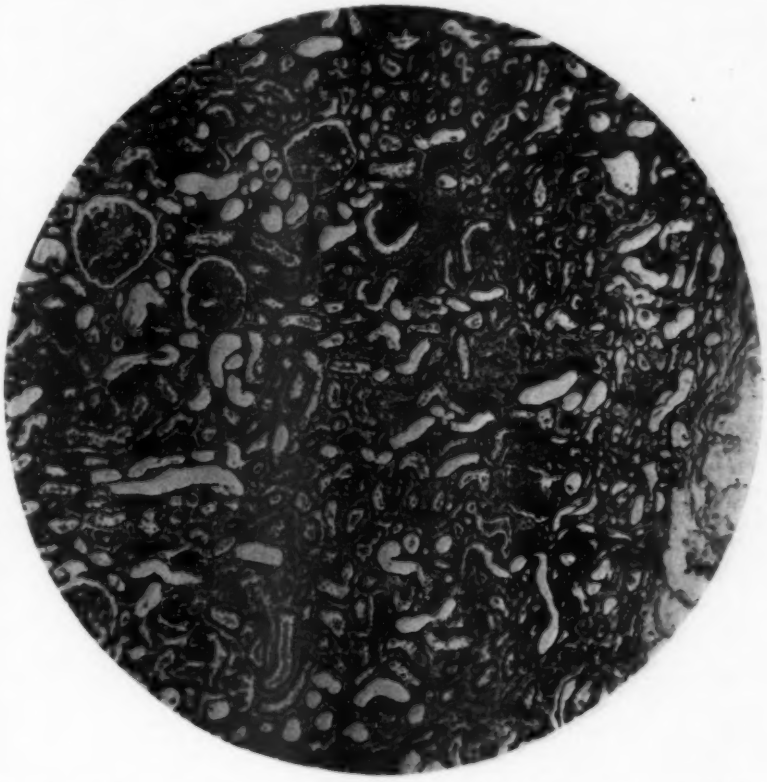

FIG. 1. LOW POWER MAGNIFICATION OF THE KIDNEY. HEMATOXYLIN AND EOSIN.

Note distension of tubules, swollen tubular epithelium and small patches of fibrosis. Glomeruli show little change. × 67.

tubules, interstitial tissue and some of the glomeruli showed the presence of many fat droplets. This fatty change, implicating the tubules and the interstitial tissue, was the most striking feature of the picture.

A series of lipoid and fat stains were employed in an attempt to determine the

The lipoid substance which was most abundant in the collecting tubules, in the loops of Henle and in the interstitial tissue, stained orange with Scharlach R, dark blue and dark purple with Nile Blue Sulphate and not at all with Neutral Red, Osmic Acid or Lorraine Smith-Dietrich. Occasional small red staining droplets were

encountered in very thin sections stained with the Nile Blue Sulphate, but these were all located in the lumina of the proximal convoluted tubules, and the bulk of the reaction was dark blue. The Golodetz reaction for cholesterol was employed without result. Under the polarizing microscope an occasional doubly refractile crystal was seen, but in no case did the distribution of these crystals bear any definite relationship to the areas in which the fat was found.

Microscopic examination of the liver showed a marked lipoid infiltration about the central zone of the liver lobule, and this lipoid material reacted with the special stains in the same manner as did that in the kidney.

None of the large lipoid cells described in other cases of cholesterol-ester infiltration were encountered.

From these microchemical studies of the tissues we believe that the type of lipoid found in the kidneys and in the liver is not a simple cholesterol-ester, but more probably belongs to the complex phosphatid group in which cholesterol is loosely bound.

The pathological diagnosis on the kidney was chronic interstitial nephritis with lipoid infiltration.

The microscopic picture of these kidneys resembles slightly that of the so-called "myelin kidney" recently studied by McNee (8). The striking whitish semi-translucent appearance of the "myelin kidney," according to McNee, is produced by a special form of fatty degeneration in which lipoid (cholesterin-ester) fat is present in large quantities. A similar microscopic picture is described by Heusler (5) who speaks of a "xanthomatosis of the kidney" in his case.

The most fundamental question in chronic nephrosis is whether the disturbance in cholesterol metabolism is primary, leading later to lesions of the kidney, or whether these cases begin as a chronic nephritis, the picture of which is later complicated by cholesterol infiltration of the kidney and disturbances in cholesterol metabolism. The patches of interstitial nephritis in the kidneys of our patient may have been secondary changes following the cholesterol infiltration.

There are many features of chronic nephrosis which suggest that it is primarily a disturbance of metabolism. This conception of the disease is stressed by Epstein and discussed by several writers. In our patient the presence in the liver of an intense lipoid infiltration similar to that seen in the kidney, is suggestive evidence that we are here dealing with a metabolic disease. The solution of this question, however, awaits the results of further investigation, particularly those upon the rôle of cholesterol in normal and in disordered metabolism.

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An Adenomyoma of the Vesico-Vaginal Septum and a Supernumerary Ovary

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As far back as 1860 Rokitansky (1) described a tumor of the uterus made up of glandular elements in myomatous tissue. Until 1896, however, when von Recklinghausen's "Die Adenomyome und Cystadenome der Uterus und Tuberwandung" (2) appeared, medical literature contained only very scattered reports of these tumors. The best known of these reports were by Babes (3), Breus (4), Schroeder (5), Schatz (6) and Diesterung (7).

Von Recklinghausen's work was so much more extensive than anything that had yet appeared on this subject and his contention that the epithelial elements in adenomyomata were embryonic remnants of the Wolffian body and duct aroused such a controversy that from that time on medical literature teemed with articles on these tumors. At first these for the most part consisted in discussions of etiology, some authorities, as Pick and Pfannenstiel, agreeing with von Recklinghausen's ideas, while others absolutely refused to accept them.

To Cullen (8, 9) belongs the credit of being the first to insist that the epithelium in these tumors is derived from the mucous membrane of the uterus, and today this contention of his, in spite of the tremendous amount of opposition with which it was at first received, is universally accepted everywhere. Cullen's first investigations were carried out on diffuse uterine growths. In these he was able to demonstrate by serial sections that in all his cases there was, at some point, a definite connection of the uterine mucosa with the adenomyoma and therefore suggested that direct extension of uterine mucosa explained the presence of the epithelial elements in all adenomyomata.

Naturally, however, inasmuch as tumors containing uterine mucosa continued to be found in more and more diverse situations, the idea that direct extension of the uterine mucosa accounted for the formation of all adenomyomata came to be modified. In 1917 Cullen himself (10), in discussing the origin of adenomyomata of the recto-vaginal septum, states: "We know nothing as to the origin of these tumors, but it is certain that their glandular elements are identical with those of the body of the uterus."

In an article published in 1921 (11), on the relation of chocolate cysts of the ovary to pelvic adenomata, Sampson threw an entirely new light upon the etiology. Later, through a

series of splendid articles (12, 13, 14), each one adducing more proof in support of his ideas, he demonstrated to the satisfaction of, at least, the majority of the gynecologists of today that he has found the explanation for the occurrence of uterine epithelium in situations far distant from the uterus itself, and has thus forged another link in the chain of investigations which have led to the understanding of adenomyomata.

Sampson's ideas may be summed up briefly as follows: The epithelium in all adenomyomata is, as Cullen has long contended, derived from uterine or tubal mucosa. This mucosa in the tumors found close to the uterine or tubal cavities has reached its position by direct extension, and these tumors Sampson calls *primary adenomyomata*. However, to quote from his article published in 1922 (13), "In the second type of growth, which in my experience is much the more frequent variety, the uterine or tubal wall is invaded by epithelium, implanted on its peritoneal surface, which is derived from the perforation of an ovarian hematoma of endometrial type or from or through the fimbriated extremity of the tube." In his last article (14) published in March 1924, he emphasizes the importance of benign and malignant endometrial implants in the peritoneal cavity and again declares his belief that the glandular element in these tumors is uterine mucosa poured out through the fimbriated end of the Fallopian tube. He further states that the uterine mucosa has either at once been deposited on the pelvic peritoneum or has, so to speak, been incubated in the ovary as a chocolate cyst of endometrical type and then extruded into the peritoneal cavity when this cyst ruptured. As many of these tumors of the second type found away from the uterus show only epithelium and no myomatous tissue, it is probably better in speaking of them, to use Sampson's term *endometrial implant* rather than to call them all adenomyomata.

After the attention of surgeons has once been directed to adenomyomata, tumors containing uterine mucosa were from time to time described as occurring in new situations. In 1896, Cullen (15) reported the first case of adenomyoma of the round ligament. In 1899, Russell (16), found in an ovary large islands of uterine mucosa. By 1920 Cullen (17), in addition to having encountered many instances of adenomyoma of the body of the uterus, had found uterine epithelium in the recto-vaginal septum, uterine horn, Fallopian tube, round ligament, ovary, utero-ovarian ligament, utero-sacral ligament, sigmoid flexure, rectus muscle and umbilicus. Mahle and MacCarty (18), writing from the Mayo Clinic, reported two cases of adenomyoma of the groin not connected with the round ligaments, as well as a number of adenomyomata in many of the localities already mentioned.

The case which I am about to report, while presenting several other features of interest, emphasizes another location where ectopic uterine epithelium may be found, namely, in the vesico-uterine space. In search ing for other instances of such cases, I find no mention in Cullen's writings.

in Mahle and MacCarty's report from the Mayo Clinic, or in Cuthbert Lockyer's splendid article (19), of the finding of uterine mucosa in this situation. The four articles on endometrial transplants published by Sampson between 1921 and 1924 represent the results of a careful study of over a hundred specimens and contain detailed reports of over thirty cases. In his Case 20 reported by Sampson in an article in the *Archives of Surgery*, September, 1921, the patient had a perforating hemorrhagic cyst of the right ovary, cancer of the uterine cervix and multiple pelvic adenomata of the endometrial type. Among these implants there was one in the vesico-uterine fold of peritoneum just beneath the left round ligament and another in the same fold just to the right of the median line. It is of interest to record that "there were no symptoms referable to the pelvic adenomas." In his next series of cases reported in November, 1922, in the *American Journal of Obstetrics and Gynecology*, we find that in Case 4 the patient had "Multiple small implantation adenomas (of endometrial type) of the posterior surface of the uterus, posterior culdesac, anterior surface of the left broad ligament and in the anterior culdesac, leiomyoma of the uterus, evidence of stromal hemorrhage in the right ovary." In this case, also, we note that there were no symptoms referable to the pelvic adenomas. In Sampson's latest publication in March 1924 (*Surgery, Gynecology and Obstetrics*), implants were encountered "on all the structures in the pelvic cavity excepting the omentum and the bladder." The following is a summary of my own case:

Miss L. B., aged forty-three, was seen July 3, 1924. At this time she was complaining of dysuria and frequency of urination. The family history and the general past history had no bearing on her present trouble. The flow had increased slightly in amount in the past few mouths, but otherwise the menstrual history was entirely normal, the periods coming at intervals of four weeks and lasting three days. There had been no history of intermenstrual bleeding at any time. The urinary symptoms of which the patient complained had been present for two years, but had become much worse in the past few months. They were of a rather unusual nature. For three weeks out of every month the patient was perfectly comfortable. Beginning about two days before the period and continuing for the three days of the period there was a feeling of discomfort in the lower abdomen, severe pain on voiding and a constant feeling of urgency causing the patient to urinate many times an hour. Haematuria had never been present. A day or two after cessation of the period the urinary symptoms would entirely disappear. This monthly recurrence of symptoms had been going on for two years but had been very much more marked in the last few months.

Examination. The findings of interest were the following: In the lower abdomen there was felt a hard symmetrical tumor which arose from the pelvis and extended to within 5 cm. of the umbilicus. (The presence of this tumor had been unsuspected by the patient.) On pelvic examination this tumor was found to be formed from the uterus. No abnormalities were palpated in the adnexa. The urinary examination was entirely normal. The patient was thought to have a myomatous uterus and an operation was advised.

Operation. August 6, 1924. St. Joseph's Hospital.

Under ether anesthesia a midline incision was made extending from the umbilicus to the symphysis. When the peritoneal cavity was opened, the lower abdomen presented the following picture. The fundus

of the uterus was about three times the natural size and had been converted into a myomatous tumor. On the right, a loop of small intestine was adherent to a cyst which arose from the anterior surface of the broad ligament. This cyst was dark brown in color and about 6 cm. in diameter. It was separated from the right ovary by the Fallopian tube and the broad ligament. There was a small dark-colored area in the right ovary and a similar area in the right Fallopian tube. (These areas are described in detail in the pathological report.) The small intestine was separated by sharp dissection from the cyst. The adhesions between these two structures were so firm that, during the separation, the cyst ruptured, emitting about an ounce of dark brown material. After the small intestine had been released from the pelvis, it was seen that the cyst had arisen from the anterior surface of the broad ligament quite close to the point at which the round ligament is inserted into the uterus. A firm grey structure was also found situated on the anterior surface of the broad ligament quite close to the point at which the brown-colored cyst had arisen.

As the patient was forty-three years of age, it was decided to do a supra-vaginal hysteromyomectomy and remove both tubes and ovaries. The round and infundibulo-pelvic ligaments were then ligated. In an attempt to strip the peritoneal reflection of the bladder downward, the vesico-vaginal septum was found to be greatly thickened and infiltrated with chocolate-colored material presenting the typical gross picture of adenomyoma. It soon became evident that the adenomyomatous tissue had invaded the wall of the bladder and that to remove the entire adenomyoma a portion of the bladder would have to be resected. Since the patient's symptoms had all been urinary in character, a radical procedure was decided upon. A panhysteromyomectomy was, therefore, done and the portion of the bladder invaded by the adenomyoma was removed. In sewing up the bladder, first the base and then the superior surface had to be sutured, for quite a large part of the bladder had been removed. In suturing the posterior wall great care had to be used in introducing the suture to avoid closing off or kinking the ureters, for on each side the resection had gone quite close to the ureter and it had been necessary to remove a diamond-shaped area of the trigonum, which included a large part of the so called inter-ureteric ridge. After the posterior wall had been sutured, the three orifices (the two ureteral and the urethral), which normally lie in such relation to each other as to form a triangle, were now situated in a line one above the other, the right ureter entering the bladder above the left.

The vaginal cuff was then brought together with two layers of interlocking stitches so as to exclude the development of a urinary fistula. Two abdominal drains were introduced, walling off, as much as possible, the pelvis from the rest of the abdominal cavity.

The patient returned from the operating room in a somewhat shocked condition but rallied promptly. The two abdominal drains were removed on the eighth day and for about ten days later there was some urinary drainage through the abdominal wound, but on the 25th day after the operation, the patient was discharged, with the incision perfectly healed and in very good general condition.

PATHOLOGICAL REPORT

The specimen consists of the entire uterus, both Fallopian tubes, both ovaries and a portion of the urinary bladder. The uterus measures 15 x 12 x 9 cm. The upper portion has been converted into a hard globular tumor, the surface of which is smooth and glistening. A little above the junction of the body of the uterus with the cervix, the anterior surface of the uterus becomes darker in color and here it is that we find the portion of the bladder which was resected.

A median section was made through the uterus and the attached bladder wall (Fig. 1). The tumor in the fundus of the uterus presents, for the most part, the typical whorl-like appearance of a myomatous growth. In one area, there is a small accumulation of old blood suggesting an adenomyomatous condition, but as microscopic study of this area fails to show the presence of uterine mucosa, this cannot be proven. The median section of the lower

portion of the fundus and of the cervix presents a very interesting condition. Well away from the uterine canal, there are two cysts filled with chocolate-colored material; the larger of these two measuring in diameter 15 x 7 mm., the smaller, 7 x 5 mm. The larger of these cysts extends to the anterior surface of the uterus: it is adherent to and has apparently extended into the bladder wall. Microscopic sections show these cysts to be lined with typical uterine epithelium and the contents of the cysts to be old blood (Figs. 2 and 3). While the vesico-vaginal septum has been invaded by the adenomyoma, the growth has not extended through the bladder mucosa. (If this had happened, with every menstrual period the patient would have passed blood through the urethra.)

The cyst which was found adherent to the anterior surface of the right broad ligament was ruptured at operation and is now represented simply by a thin-walled sac. As is well shown in Figure 4, which is a drawing of conditions found at operation, this cyst arose from tissue on the anterior surface of the broad ligament in front of the Fallopian tube. Microscopic sections from the walls of this cyst show typical luteum tissue such as is seen in any corpus luteum cyst of the ovary. Lying beside this collapsed cyst, there is a little structure, measuring 12 x 8 x 5 mm., which is attached to the anterior surface of the mesosalpinx by a small but definite pedicle. This structure and the collapsed cyst both lie in front of the Fallopian tube and are in no way connected with the normal ovary on that side. The external surface of this structure is grey in color and has a somewhat puckered appearance. Microscopic sections of this little body show typical ovarian tissue with Graafian follicles, corpora albicantia and typical stroma (Fig. 5).

The right ovary measures 3.5 x 3 x 1 cm. It appears to be entirely normal except for a bluish black area, 4 mm. in diameter, on its lateral under surface which suggests

FIG. 1. MEDIAN SECTION OF ENTIRE UTERUS AND CERVIX
Arrows point to the two endometrial cysts (adenomyomata) which involved the bladder wall.

one of the haemorrhagic ovarian cysts or endometrial transplants so often described by Sampson. Microscopic sections of this area (Fig. 6) show a dilated cyst lined with very low epithelium. No old blood is present. Possibly we are dealing with uterine epithelium changed by pressure, but the that it contains a definite lumen lined with columnar epithelium (Fig. 7). We have here probably an accessory tubal ostium, a condition encountered fairly commonly.

The only findings of interest on examination of the left adnexa are a small corpus luteum cyst in the ovary and a hydatid of

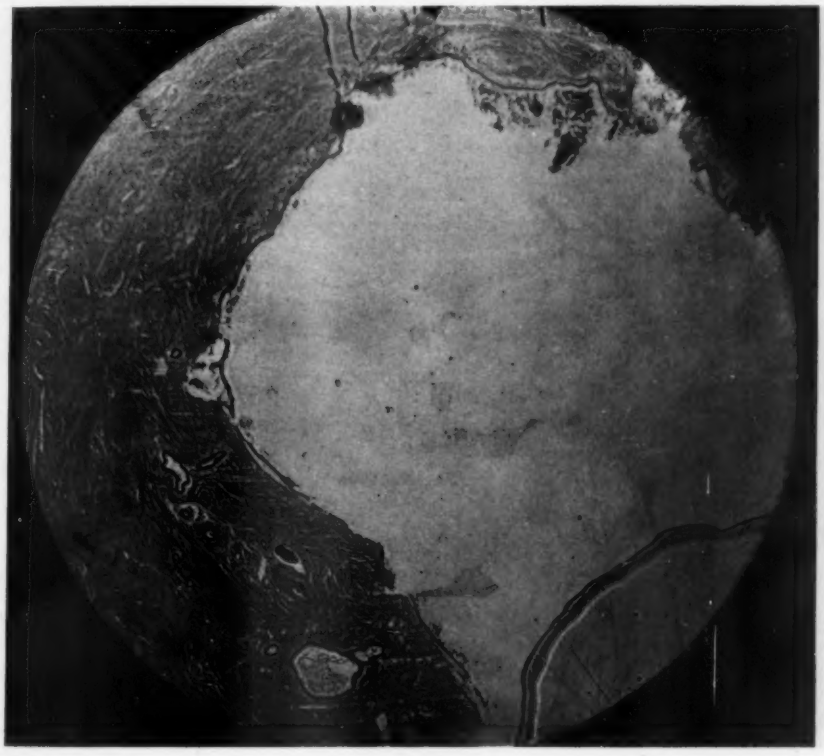

FIG. 2. LOW-POWER PICTURE OF ADENOMYOMA

In the upper part of picture old blood is seen in the large cavity. Lower part of picture shows columnar epithelium and glands such as are seen in the body of the uterus.

picture is not typical enough to warrant the statement that it is an endometrial transplant.

The right Fallopian tube measures 10 cm. in length. The fimbriated end is open. Near it, on the peritoneal surface, there is a bluish nodule, 2 mm. in diameter (Fig. 4). Microscopic sections of this nodule show Morgagni attached to the broad ligament just below the distal end of the Fallopian tube.

Before concluding the report of this case a short summary of the literature on aberrant ovarian tissue

may be of interest. The first mention of this condition seems to have been by Grohe (20) in 1864, who reported finding at autopsy three ovaries in a woman forty years of age. One of the ovaries was of normal size and on the right side. The other two were on the left side and were somewhat smaller than normal. Of these two, one was normally situated and attached to the uterus by a utero-ovarian ligament; the other lay on the posterior surface of the broad ligament in a reduplication of the peritoneum and was not attached to the uterus.

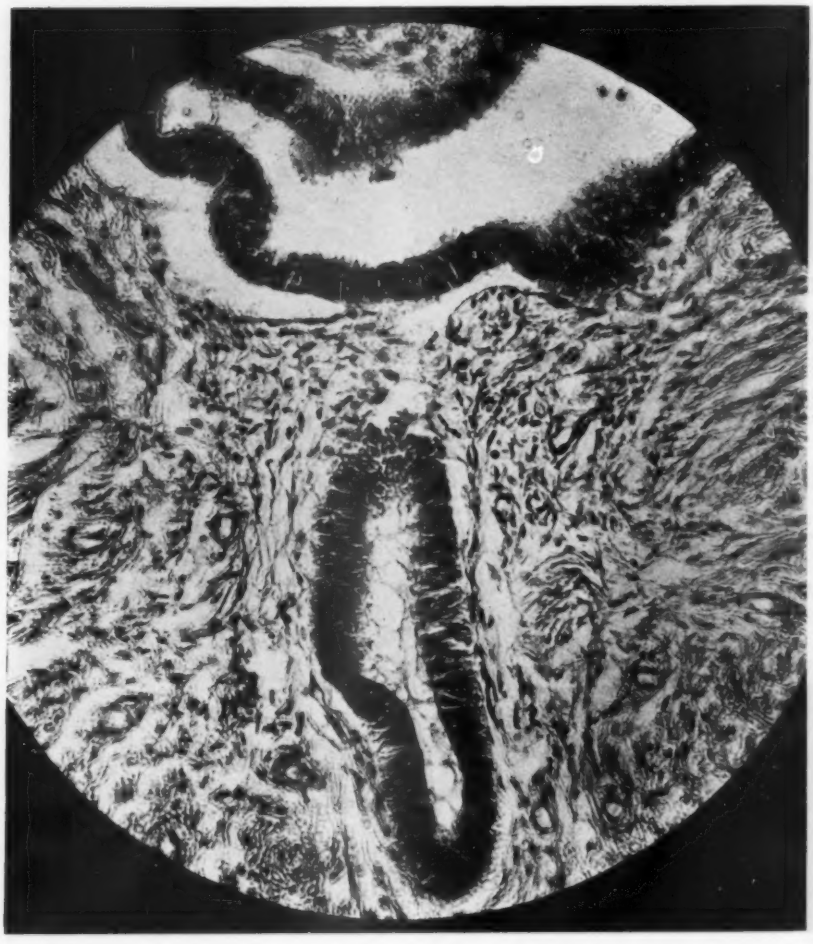

FIG. 3. HIGH-POWER PICTURE OF TWO OF THE SMALL GLANDS IN FIGURE 2

After Grohe's report other accounts of this condition began to appear in the literature, but authorities differed widely in their opinions as to the frequency of its incidence. Beigel (21), as far back as 1887, reported twenty-three instances of accessory ovarian tissue in 500 autopsies, and von Winckel (22), writing in 1890, ovary as an additional ovary;" and further on he adds: "So far as the evidence at present stands, an accessory ovary quite separate from the main gland so as to form a distinct organ has yet to be described by a competent observer." Lawson Tait (24), while having personally seen no instance of an accessory ovary, seems

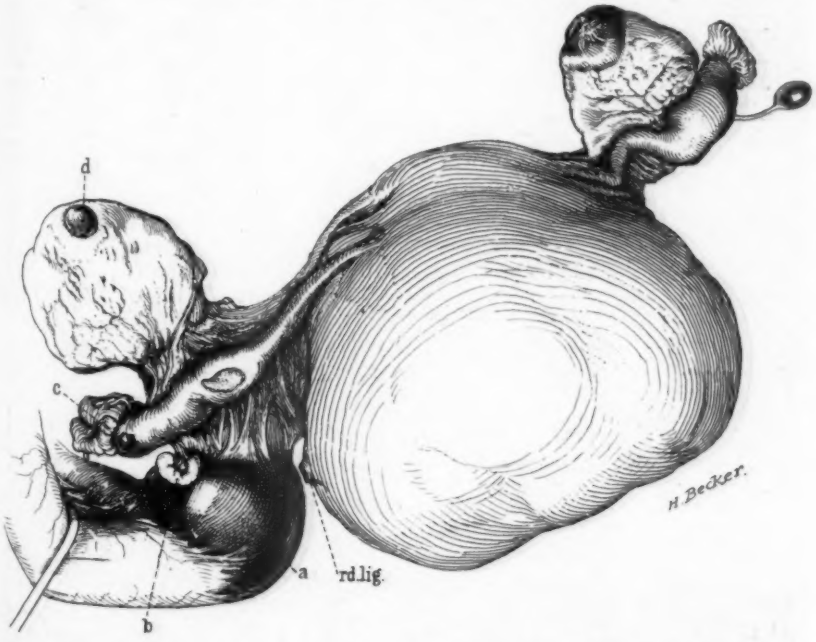

FIG. 4. SHOWS THE CONDITION FOUND AT OPERATION, AS SEEN FROM ABOVE

a, corpus luteum cyst. *b*, supernumerary ovary. *c*, an accessory tubal ostium. *d*, endometrial implant on the normally situated ovary.

in the same number of autopsies found eighteen. In contrast to this, in 1891 so great a gynecological authority as Bland Sutton writes (23): "The statement that Beigel and Winckel found this condition so commonly is sufficient to indicate that these writers must have regarded any pedunculated body near the to have taken a somewhat more favorable view of the report of the German authors, and Dudley (25), in his textbook, published in 1898, states that accessory ovaries are found in from 2 to 3 per cent of all autopsies. Seitz (26), in 1900, in a splendid article reviewed the whole subject and gathered from the litera-

ture quite a large number of unquestionable instances of accessory ovarian tissue. Madame Eisenberg-Paperin's "Contribution a l'étude des ovaires surnuméraires" (Thèse de Paris, 1911) (27) is probably the most complete work yet published on the subject. This author, although being unwilling to accept without a certain reserve some of the cases reported

FIG. 5. LOW-POWER PICTURE OF SUPERNUMERARY OVARY

Upper portion of picture shows a small Graafian follicle. Corpora albicantia in lower portion of picture.

by Beigel, because of lack of microscopic confirmation of the diagnosis, was able to collect from the literature 52 proven instances of accessory ovarian tissue. Between 1911 and 1924 seven reports have been added to the literature. Three of these described two supernumerary ovaries and will be mentioned later. The instances of a true supernumerary ovary. Von Winckel's case, reported in 1881, was the first to appear in the literature. This observer diagnosed his case as being an instance of true supernumerary ovary and thus separated it from all the other cases of accessory ovarian tissue reported up to that time. The

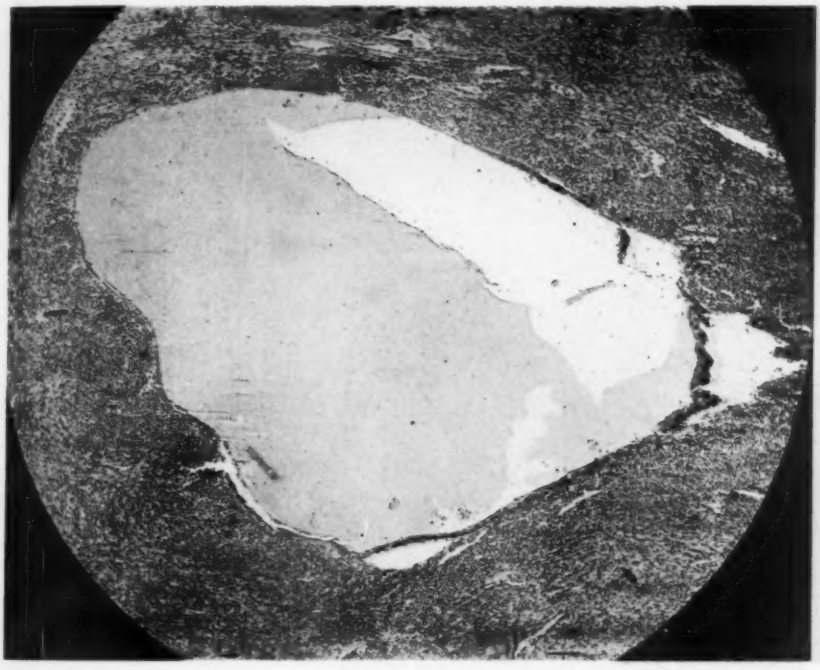

FIG. 6. LOW-POWER PICTURE OF SMALL HAEMORRHAGIC CYSTS IN OVARY
The epithelium lining the cyst appears flattened

other four articles were written by Meriel (28), Mollar (29), Latzko (30), and Smith (31).

In contrast to this relatively frequent incidence of accessory ovarian tissue, the more carefully we go over the literature the more striking becomes the extreme rarity of recorded points he made were as follows: In the first place, the third ovary, instead of being in the vicinity of the two normally situated ovaries, was attached to the anterior surface of the broad ligament; secondly, there was a definite pedicle attaching the extra ovary to the uterus; and finally,

there was no evidence around the normal ovaries of any pre-existing peritonitis which might suggest that through adhesions the third ovary had split off during the foetal or post-foetal life from one of the other two.

Seitz, in 1900, after reviewing the then existing literature on supernumerary and accessory ovaries (in all about forty articles) found that, since von Winckel in 1881 had reported his case, there were only four additional instances of a supernumerary ovary on record. These were reported by Keppler (32), Schantz (33), Falk (34) and Voigt (35), and the condition in the patients of these four authors differed from that in von Winckel's in two points; first, because there was in each instance a supernumerary Fallopian tube along with the extra ovary, and secondly, because the third ovary, while far away from the normally situated ovaries, was on the posterior instead of the anterior surface of the broad ligament. There is, however, no doubt that these cases were true examples of supernumerary ovaries.

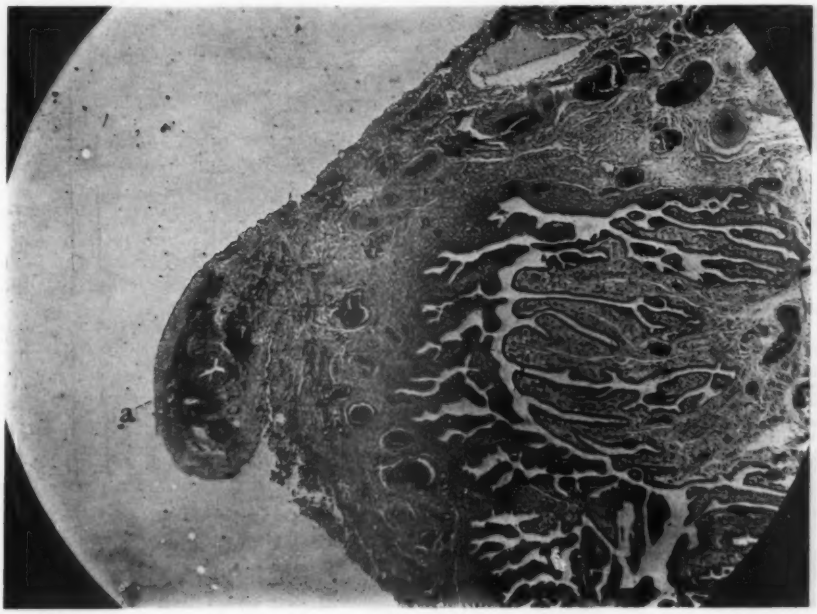

FIG. 7. LOW-POWER PICTURE OF CROSS SECTION OF RIGHT FALLOPIAN TUBE
a points to the lumen leading to the accessory tubal ostium

I have gone over all the literature on extra ovaries from the publication of Seitz's article in 1900 up to 1924. During this time there have been about thirty articles written on accessory ovarian tissue, but I have been able to find records of only three

cases of supernumerary ovaries, those of Monch (26), Liegner (37) and Furniss (38). In all three, the third ovary was well away from the normally situated ovaries and attached to the uterus by a special pedicle. However, inasmuch as the extra ovary in every case was on the posterior and not on the anterior surface of the broad ligament and in none was there a supernumerary Fallopian tube, it must be admitted that the possibility of the extra ovaries having developed through some embryological happening to the two normally situated ovaries cannot be so completely excluded as in the five original cases mentioned by Seitz in his article.

That the structure in my case which the microscopic examination showed to be made up of typical ovarian tissue was a true supernumerary ovary is proven by the fact that it was situated on the anterior surface of the broad ligament at a point so distant from the normal ovary on that side that it could not possibly have originated from it. In von Winckel's case the supernumerary ovary was on the anterior surface of the broad ligament; in all other cases except his and the one reported in this article it was on the posterior surface.

As to etiology, it was von Winckel's belief, and this belief was later held also by Engstrom (39) and then by Seitz, that, while the majority of cases of accessory ovarian tissue are the result of a division of the original ovary due to some inflammatory state arising during foetal or post-foetal life, the origin of true supernumerary ovaries is to be sought in early embryonic life when the sexual glands are differentiating from the urogenital ridge. There seems at present to be insufficient evidence at hand from which to draw any more exact conclusions.

SUMMARY

The two main features of interest to this case are the following:

1. An adenomyoma of the vesico-uterine space. Although we find in the literature two instances of endometrial implants on the anterior surface of the uterus at, on, or near, the peritoneal reflection of the bladder (Sampson's cases), this is the first case reported in which an adenomyoma in this situation has produced symptoms.

2. A supernumerary ovary situated on the anterior surface of the broad ligament. If we consider as true instances of this condition the three cases reported since Seitz's article in 1900, the total number, including my own, is still only nine. This case and the one reported by von Winckel are the only ones in which the ovary was attached to the anterior surface of the broad ligament.

In conclusion, I wish to thank Dr. Thomas S. Cullen for giving me the facilities of the laboratory of gynecological pathology of the Johns Hopkins Hospital and for his criticisms and suggestions in the preparation of this paper.

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Spontaneous Contractions of the Pregnant Human Uterus

A Preliminary Report[1]

By Ko Chi Sun

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SINCE the conception by Munde, Tyler-Smith, Löwenhardt, Beard and others of a menstrual periodicity of the uterine contractions and the demonstration of its actual existence in the albino rat by Blair (1) and in the domestic pig by Keye (2), considerable interest has been aroused on the subject, as is evidenced by the rapid accumulation of literature within a short period. In 1924 Wislocki and Guttmacher (3) placed the excised entire uterus and tubes of the sow in a warm suitable fluid and observed and confirmed, to a greater or less degree, what had previously been described. During pregnancy, the movements of the uterus have been studied by Kurdinowsky (4) and others, while Wijsenbeek and Grevenstuck (5) extended their observations to the puerperium. These observations, however, were all made upon laboratory animals, and as yet little is known as to what occurs in the human being. E. Kehrer (6) was the first to observe the spontaneous contractions in the excised human uterus, but as the organ was abnormal in both of his cases, there being a myoma in one case and a chronic pelvic inflammatory process in the other, he was in doubt whether such contractions would occur under normal conditions. Within the past few months, Flury (7) published similar observations on 13 human uteri. Accordingly, although spontaneous contractions undoubtedly occur in the isolated diseased human uterus, very little has been written concerning the normal human uterus,[2] and still less is known in regard to it during pregnancy.

The object of the present report is to call attention to certain definite and constant phenomena which were observed in 57 isolated human pregnant uteri, studied in the laboratory of the Woman's Clinic of the Johns Hopkins Hospital, between December, 1922, and September, 1924. The study was undertaken at the suggestion of Dr. Williams, who has constantly supervised the work, and it is now being expanded to include investigations into various obstetrical conditions.

MATERIAL AND METHODS

The specimens of uterine muscle were procured at the operating table when operations were performed which

[1] Received for publication, November 18, 1924.

[2] I have observed spontaneous contractions of a uterus from a six-month foetus.

necessitated opening the uterus, after laparotomy. In such cases a longitudinal strip of uterine muscle, 3 to 5 mm. thick, is excised from one edge of the uterine wound. The topography of the strip is carefully noted, for reasons which will be explained later. In classical Caesarean sections in this clinic, the incision is made in the midline of the organ, with its lower end extending to within 2 or 3 cm. of the bladder peritoneal fold. In cases of hysterectomy in which the entire uterus is available, several strips are usually excised and their location carefully noted. In such cases, as well as in the so-called low cervical Caesarean section, portions from the lower uterine segment are also obtained, which was possible in fifteen instances. The removal of the muscle strip specimen was made within eight to twenty-five minutes after the anesthesia was begun. In 8 instances, the patient received chloroform or ether, while, in 49, combinations of nitrous oxide, oxygen and ether were employed. In one case only did the patient receive atropine twenty minutes before anesthesia. Whenever feasible, pituitrin was not administered until after the specimen of muscle had been excised, so that complications incident to the use of that agent were avoided. The specimen was wrapped in a sterile dry sponge and taken to the laboratory, but when it was necessary to preserve it for later experimentation, it was placed in the ice-box in an air-tight but moist jar.

The apparatus employed in our observations was of the type generally used in the study of muscular contractions. One, or more than one, strip of muscle about 3 mm. wide and 20 mm. long was attached by one end to the extremity of a writing lever and by the other end to the hook of glass tubing through which oxygen or some other gas could readily be passed. The tubing together with the strip of muscle was immersed in Locke's or Tyrode's solution, kept at 37.5°C. by means of a water-bath or a microburner. In our early experiments glucose was added to the suspension fluid, but as it necessitated frequent change on account of the development of fermentation products, it was eventually discarded, as we found no difference in the activity of the uterine muscle when it was dispensed with. In experiments in which a comparative record of the behavior of several strips was desired (as in Figs. 4, 5, 6 and others), two or three strips of the same length and width were immersed in the same suspension solution and attached to different writing levers, so that identical conditions of temperature and pressure were assured. When it was desired to observe the effects of different agents upon muscle from the same region at the same time, several strips of the same dimensions were suspended in the same fluid of the same tonicity, but in different containers. In this event, the several levers can be so adjusted as to write simultaneously upon the same drum without difficulty. All the levers and the glass-tubing hooks were fastened to a single iron stand so that they could be lifted out of the suspension solution with one movement, whenever the latter needed to be changed, the fresh suspension solutions having been previously warmed to 37.5°C. and saturated with

oxygen or whatever other gas was being employed. In the comparative observations upon the upper and lower segments of the pregnant uterus, frozen histological sections of the latter were examined, as a preliminary, in order to insure the exact location. In none of these preparations was the cervical mucosa seen, but the lining was made up of decidua, to which in many cases the membranes were still attached.

The mode of caring for the specimens for a study of this kind is of such importance that this point can hardly be emphasized too strongly. At the operating table, there may be contact with xylol from the tubes of catgut, with alcohol, mercuric chloride or other disinfectants which might alter the activity of the uterine muscle. Even the salt solution employed at operations is not always isotonic and thus may affect our specimens. Consequently, immediately upon their excision, the specimens should be received by an assistant upon a sterile dry sponge or some other sterile dry container, and no attempt should be made to use a fluid medium to prevent drying, as it might be detrimental to the musculature. The specimen should be taken to the laboratory at once, and not placed upon an instrument table. When it is to be kept for later use, it has been found best to wrap the specimen in a sterile dry sponge and to preserve it in the ice-box in a clean air-tight but moist jar. Specimens from different individuals should not be wrapped in the same sponge lest some possible foreign protein reaction might occur. Moreover, specimens from the same individual, but excised before and after pituitrin has been administered, should be kept separately in order to avoid diffusion of the drug.

OBSERVATIONS

Observations were made on 57 human pregnant uteri, divided as follows:

One from the third week of pregnancy, but with a hydatidiform mole.

One from the sixth to eighth week.

Six from the eighth to twelfth week.

Three from the twelfth to sixteenth week.

One from the twenty-sixth week.

Forty-three from the fortieth week (term).

Two from the first day of the puerperium.

Of the patients at term, 22 were operated upon before the onset of labor, while, of the remainder, 13 were operated upon after being one to six hours in labor, 3 from six to twenty-four hours, and 5 after a longer period. Among the latter, one patient was threatened with rupture of the uterus. In the 57 specimens, muscle from the lower uterine segment was obtained for observation in 15 cases, 13 of which were obtained before, and 2 after, the administration of pituitrin.

In all but two instances, the isolated but artificially nourished muscle from the upper uterine segment showed spontaneous contractions immediately after excision, sometimes even without warmth or nursing fluid, and in many cases without oxygen. The initial contractions with or without oxygenation are small, but with a free supply of oxygen they attain greater magnitude in about two or three hours. During this period a change occurs in

the tonicity of the muscle—sometimes a decrease but generally an increase before oxygenation, but invariably an increase after oxygenation. The tonus usually reaches its high level during the third hour and remains at the same height thereafter. For this reason, it is customary not to regard the contractions before the third hour as normal, nor to proceed with any experimentation without having previously recorded acceptable normal contractions for comparison. When the temperature is maintained at a constant level of 37.5°C. and the current of oxygen flows at the rate of about one bubble per second, and when no other influences come into play, the muscle maintains its contractions of fairly constant amplitude in reasonably varying form and with fairly regular rhythm for a long period—one specimen having contracted continuously for sixteen hours. In this particular instance, only one change of the suspension fluid was necessary, but this interruption, though lasting only forty seconds, altered the contractions for the next thirty minutes. As a

FIG. 1. SPONTANEOUS CONTRACTIONS OF A UTERUS AT TERM LASTING SIXTEEN HOURS
Experiment begun thirty-two hours after its excision

FIG. 2. SPONTANEOUS CONTRACTIONS OF A UTERUS AT TERM AFTER SEVEN DAYS IN ICE-BOX

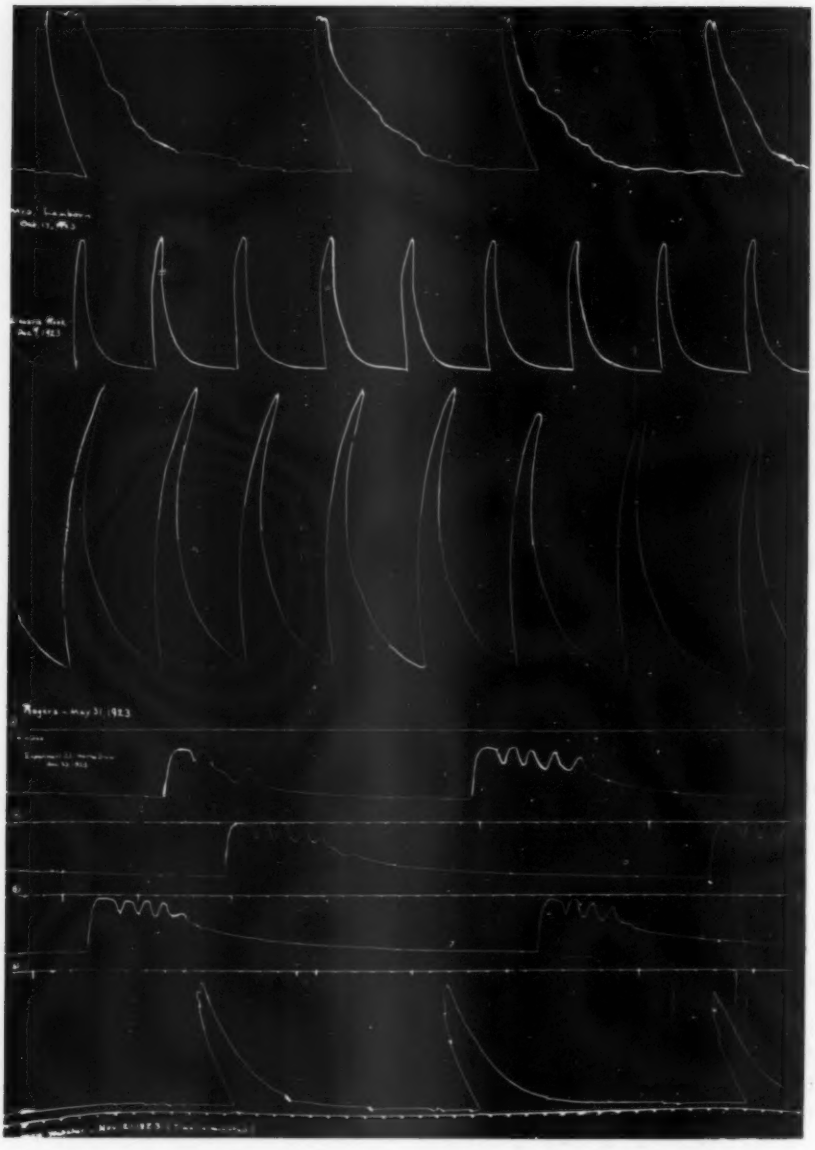

FIG. 3. SPONTANEOUS CONTRACTIONS OF UTERI AT VARIOUS PERIODS OF PREGNANCY

A, six to eight weeks. *B*, ten weeks. *C*, twelve weeks. *D*, twenty-sixth week. *E*, at term before onset of labor.

rule, the contractions towards the end gradually become smaller and less frequent and finally disappear with a marked but gradual fall of the tonus. Microscopically, the muscle becomes swollen, partially macerated, and emits a distinct odor. When hardened, cut and stained with haematoxylin and eosin, its histological appearance is, however, indistinguishable from that of normal muscle.

The spontaneous contractions, as well as the tonicity, of the muscle were observed in all specimens from the upper segment of the uterus in all stages of pregnancy. The former is so inherent in the pregnant uterine musculature that the contractions not only commence without nourishment or warmth, but also occur equally well even after the specimen has been kept in the refrigerator for six or seven days. The same applies to the persistence of tonicity which is astonishingly marked, irrespective of the age of the specimen. Figures 2 and 12 show the contractions of one specimen immediately after and many hours after the operation. Moreover, we have observed no characteristic difference in the duration of contractions of specimens obtained from uteri at various periods of pregnancy. Accordingly, such contractions appear to be a persistent property of the pregnant uterus, provided the specimen is well cared for. It is, therefore, to be expected, and we find it true, that the duration of the contractions becomes progressively less the longer the specimen has been preserved.

The character of the contractions is the most variable feature in our findings. It is different not only in uteri of varying stages of pregnancy, labor and the puerperium, but also in specimens from the same uterus if the preparations are taken from different regions or from different layers, or even if they are cut in a different plane. Unlike those of the rat and the pig, whose uterine walls are thin and uniform in structure, the contractions of preparations from the thick-walled human uterus, with its complicated structure, can scarcely be expected to be uniform. Corner has already found it difficult to secure a uniform wave of contraction in the uterus of Macacus rhesus; consequently it seems unlikely that a normal wave will be established for human uterine contractions.

The difference in the contraction wave from specimens obtained from the several layers of the uterus is very striking. In general, those of a strip from the subperitoneal layer consist of a straight wave in the form of an inverted V, with or without superimposed minor waves of retraction. Those from a subdecidual strip present similar waves, but more frequently superimposed, as well as interposed, by small or large retractions; whereas a strip from the middle layer has such bizarre types of contraction that their description is impossible. After a long period of contraction, the waves may be represented only by tiny humps.

The frequency of the contractions appears to depend almost entirely upon the location in the musculature from which the specimen has been taken. Slight variations, however, exist during the various periods of pregnancy, the contractions becoming more frequent from the second month onward, slower towards the middle of

FIG. 4. COMPARATIVE RECORDS OF SPONTANEOUS CONTRACTIONS OF DIFFERENT LAYERS OF THE UTERUS
Experiment 50—sixteenth week. Experiment 111—at term before onset of labor. Experiment 101—at term after onset of labor.

FIG. 5. COMPARATIVE RECORD OF SPONTANEOUS CONTRACTIONS OF DIFFERENT "ZONES" OF UTERUS AT TERM

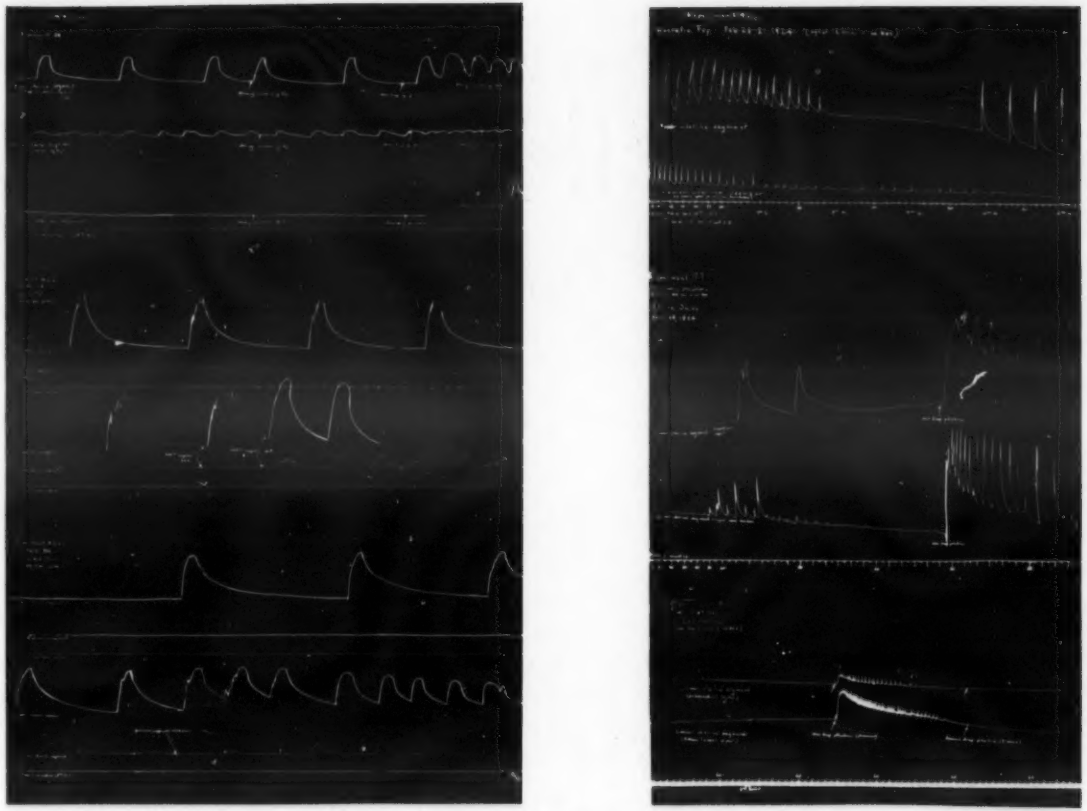

FIG. 6. PASSIVE CHARACTER OF THE LOWER UTERINE SEGMENT AND ITS CONTRACTIONS AFTER ADDITION OF PITUITRIN OR OTHER DRUGS

Experiments 39, 77, 53—at term. Experiment 42 a, b, c— ten to twelve weeks. Experiments 42 c, and 53—addition of pituitrin in vivo. Experiment 77—pituitrin given before removal of specimen.

pregnancy, and again somewhat more rapid towards term. Unfortunately, I have no observations on normal specimens from the twenty-sixth week to the thirty-seventh week of pregnancy and therefore cannot show at present just when the changes in rate of the contractions occur. The one specimen from the middle of pregnancy was, however, complicated by the premature separation of the normally implanted placenta, although the particular strip of muscle experimented upon showed no haemorrhagic dissociation of its fibres. Next, if the total thickness of the slab is divided into layers, the subdecidual contracts more frequently than the subperitoneal layer. So also, if a long strip of muscle is cut into segments, each segment will show contractions of a different rate. Hence, it is possible to divide the uterus into different zones, according as the contractions occur more or less frequently. The dividing line is formed by Bandl's contraction ring. Above this, or in the upper uterine segment, spontaneous contractions occur, while below it, or in the lower uterine segment, no spontaneous contractions have been observed. Within the upper segment contractions occur in more rapid succession in the neighborhood of the fundal region, but become less frequent as the site of Bandl's ring is approached. On one occasion strips of muscle were excised from the right and the left half of the uterus and our observations showed that the contractions from the right half were faster and more vigorous than those from the left, but at the same time the shape of the waves of both were surprisingly similar.

The discovery of the characteristic behavior of the lower uterine segment began the study of the behavior of specimens obtained at so-called cervical Caesarean sections, and, as has already been said, the muscle from this region does not contract spontaneously. Upon the addition of pituitrin, however, an immediate response results, when the contractions may be tetanic for a short while, and then assume the same rhythmic character as the upper segment. When ergotol or quinine sulphate was added, no tetanic contractions were observed, but both of these drugs gave rise to rhythmic contractions which persisted for several hours. This phenomenon has been so uniformly and repeatedly observed that one became curious to know what the lower segment would do if pituitrin were given before the specimen were excised. Accordingly, this was done in 2 cases, and in both of them the preparations from the lower segment contracted without the addition of pituitrin to the suspension fluid. It was also noticed that when the lower segment contracts, the rate of contraction is faster in its subdecidual than in its subperitoneal layer.

In addition to studying specimens obtained at Caesarean sections upon women presenting no clinical abnormality apart from disproportion between the size of the head and the pelvis, I also had an opportunity to study the uterine muscle from several patients in whom the clinical course of labor presented unusual features. The observations have been of great interest, and have served to give a rational explanation for certain clinical peculiarities of the cases. For example, the first observation was made on a specimen obtained from a primipara

pituitrin in vivo. Experiment 77—pituitrin given before removal of specimen.

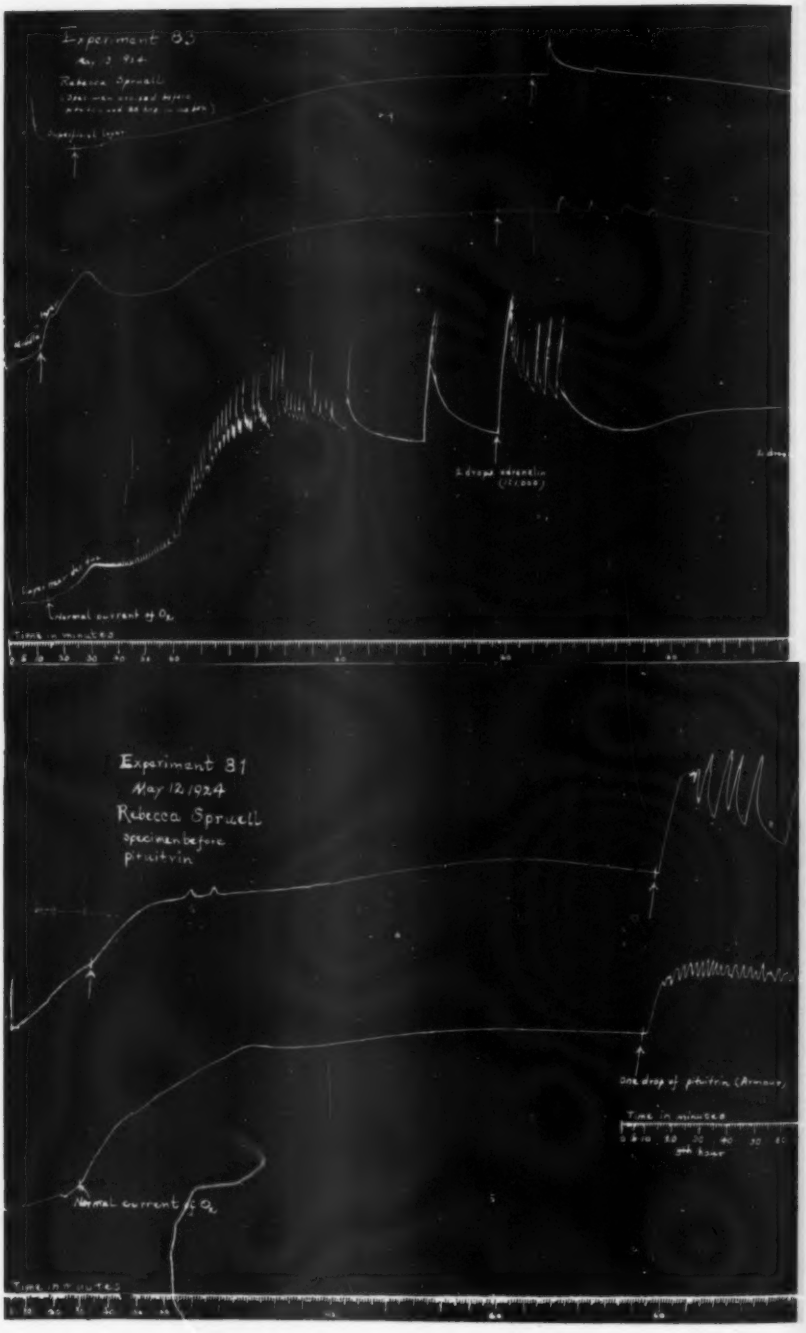

FIG. 7. SECONDARY UTERINE ATONY

who was admitted after three days of hard labor with a tetanically contracted uterus and all the signs of threatening rupture. At first glance, the tracings apparently showed that the uterus was contracting so poorly that the waves appeared only as short humps. But after more extended experience in the interpretation of the tracings, it appears that these humps indicated a tetanic type of contraction, similar to that induced by pituitrin or to the worn-out type of wave which may follow long continued spontaneous contraction. In other words, the tracings gave visual confirmation of the clinical findings, and show that the normal intermittent contractions had given place to a continuous tetanic state (Fig. 9).

Furthermore, in May, 1924, I obtained a specimen from a multiparous patient, who presented the following history. After being in labor for three days, the pains completely ceased, and were entirely absent when she was admitted twelve hours later. As signs of intra-partum infection were present, the uterus was amputated after the Caesarean section, so that abundant material was available for experimental purposes. When strips of muscle were suspended in oxygenated Locke's solution, they failed to show any sign of activity during an observation lasting seven hours. When pituitrin was added to the solution, typical contractions occurred. This observation clearly demonstrates that the uterus had passed into a condition of atony, but that its muscle was still susceptible to the stimulating action of pituitrin (Fig. 7).

A third case was of still greater interest. In this instance, the third pregnancy of a thirty-year-old woman was ended by Caesarean section on account of the following history. She had been attended by a member of the staff in her two previous labors, each of which was prolonged on account of weak and infrequent contractions, and was eventually terminated by the forceps delivery of a dead child. Moreover, a sister had had an almost similar experience, and the patient stated that all the labors of her mother had been unusually prolonged on account of defective pains. Observations upon her uterus are illustrated by Figure 8, and show irregular and feeble contractions similar to those noted in muscle which has been preserved in the ice-box for a week. This behavior apparently justified delivery by Caesarean section, as it is probable that without it the present pregnancy would have ended in the same way as the other two.

In view of the type of contractions shown by the tracings, and the past history of the patient, as well as that of her sister and mother, Dr. Williams suggested that the condition might appropriately be designated as "familial uterine inferiority."

Furthermore, I was able to study the behavior of the uterine muscle from 3 patients upon whom Caesarean section had been done for premature separation of the normally implanted placenta. I was greatly surprised to find that the muscle contracted in the first observation, which appeared improbable from what we know concerning the lesions in that condition. Further investigation, however,

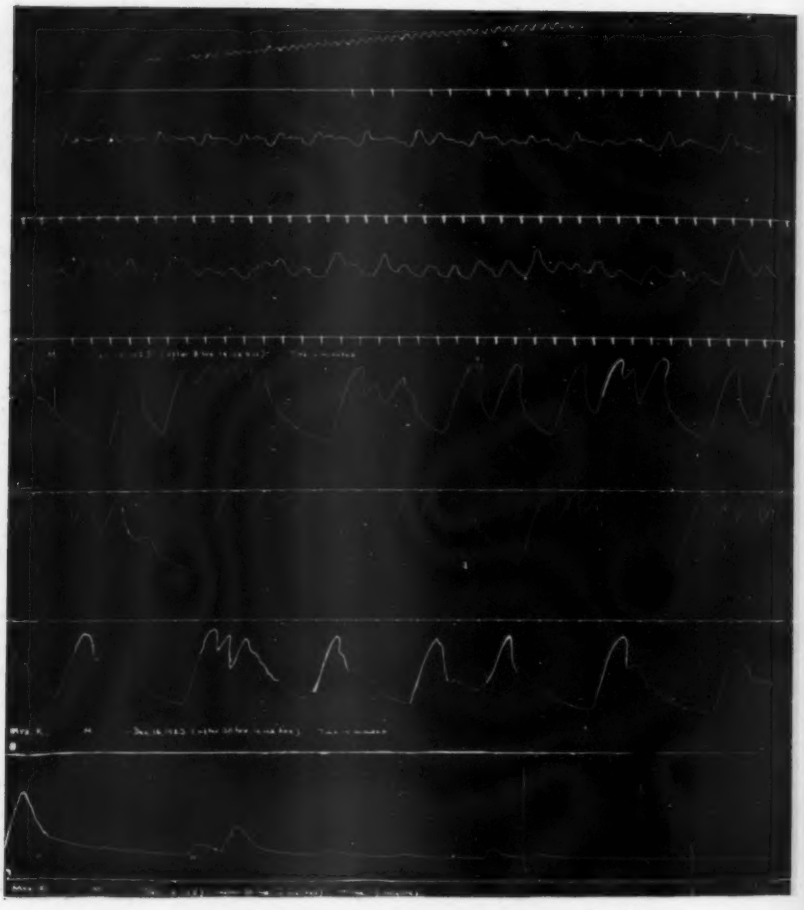

FIG. 8. "FAMILIAL UTERINE INFERIORITY"

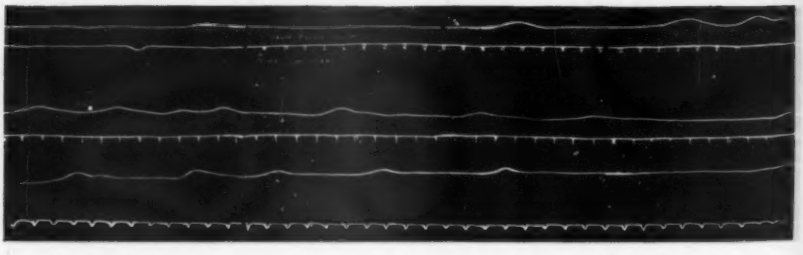

FIG. 9. TETANIC STATE OF UTERUS AFTER THREE DAYS OF HARD LABOR

showed that the characteristic dissociation of the muscle fibres was limited to the posterior, while the muscle strip had come from the anterior wall of the uterus, which was therefore essentially normal.

In the other 2 cases, specimens were obtained from hemorrhagic as well as from non-hemorrhagic areas, with the result that the corresponding tracings showed striking differences. Those from the hemorrhagic areas showed a

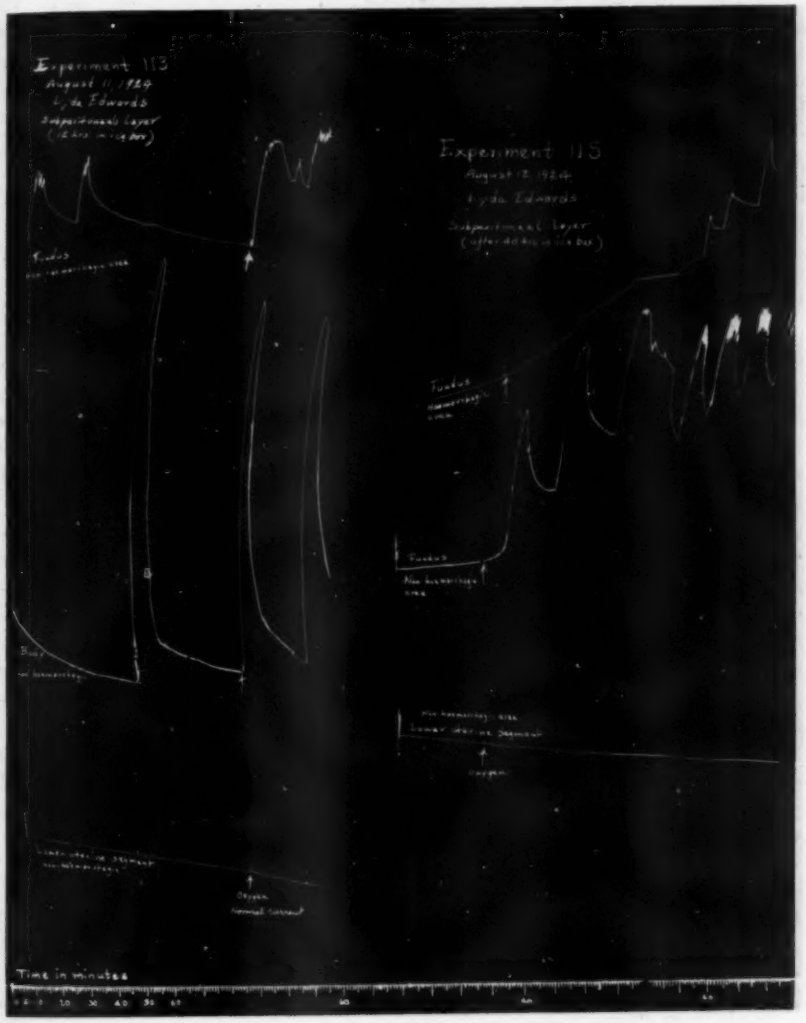

FIG. 10. PREMATURE SEPARATION OF NORMALLY IMPLANTED PLACENTA

tetanic or refractory tendency, although once in a while a step-like contraction occurred. On the other hand, those from the non-hemorrhagic areas presented characteristic contractions, which tended to become more irregular and less rhythmic than in the normal organ.

In studying specimens from the puerperal uterus, it was not surprising to find that they exhibited contractile activity. In the two uteri observed, the subdecidual layer showed a condition of tonus, but did not alternate between contraction and relaxation. Whether this represents an actual tetanic state requires further observation, as will be pointed out later.

In addition to our studies of the normal action of the muscle, some 70 pharmacological experiments were made by adding various drugs and protein material to the suspension fluid, but as yet characteristically uniform results have not been obtained. I have already referred in a cursory manner to the effects of pituitrin, ergot and quinine, but shall defer their detailed consideration to a future communication. I might, however, say in a general way that the published pharmacological action of various drugs upon the uterus is not substantiated by the behavior of the normal human organ. Furthermore, in view of the difference in behavior of the several portions of the uterus, the results obtained can not be considered as specific unless one is sure that corresponding portions of the uterine musculature have been employed in the various experiments. It should also be borne in mind that erroneous conclusions may be drawn if the various agents are added to the suspension fluid before the contractions have had time to assume their normal form.

DISCUSSION

From what has been said, it is apparent that the results which we have obtained are very individualistic, and may depend upon inherited tendencies, as well as upon the varying stages of pregnancy, labor or the puerperium, at which the specimens were obtained. Furthermore, it has been shown that the character of the contractions will vary according to the various portions of the uterine wall from which the specimens are obtained. For these reasons, I shall summarize my findings quite briefly, and shall attempt to bring them into correlation with the findings at the bedside.

It would seem justifiable to conclude that the rhythmic contractions of the pregnant organ should be identified with the Braxton Hicks contractions, which may be observed clinically in all pregnant women. Our tracings show that they have a regular rhythm and occur more frequently during the early part of pregnancy, to become slower later, but tend to revert to their original frequency as term is approached. In the puerperal uterus, similar contractions of lesser frequency are observed, and it is probable that they may account for the so-called "after pains."

In all instances the contractions are most vigorous in the body of the uterus, become less well marked as the lower uterine segment is approached, and are entirely lacking in the latter. This lack of contraction may be regarded as a constant phenomenon,

and was noted in the thirteen specimens of lower segment obtained from patients who had not received pituitrin prior to the excision of the muscle strip. It may be regarded as affording experimental evidence of the clinically passive behavior of the lower uterine segment. Moreover, the behavior of this segment following the use of pituitrin affords an explanation for the occurrence of rupture of the uterus after the administration of

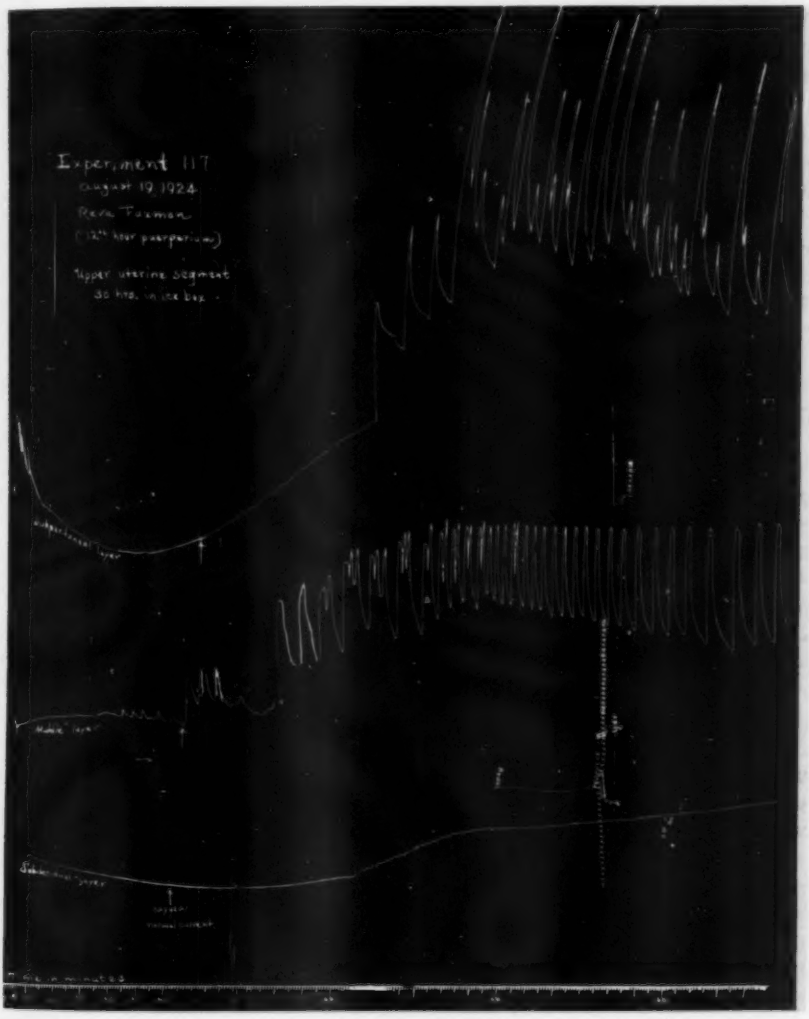

Fig. 11. Puerperal Uterus

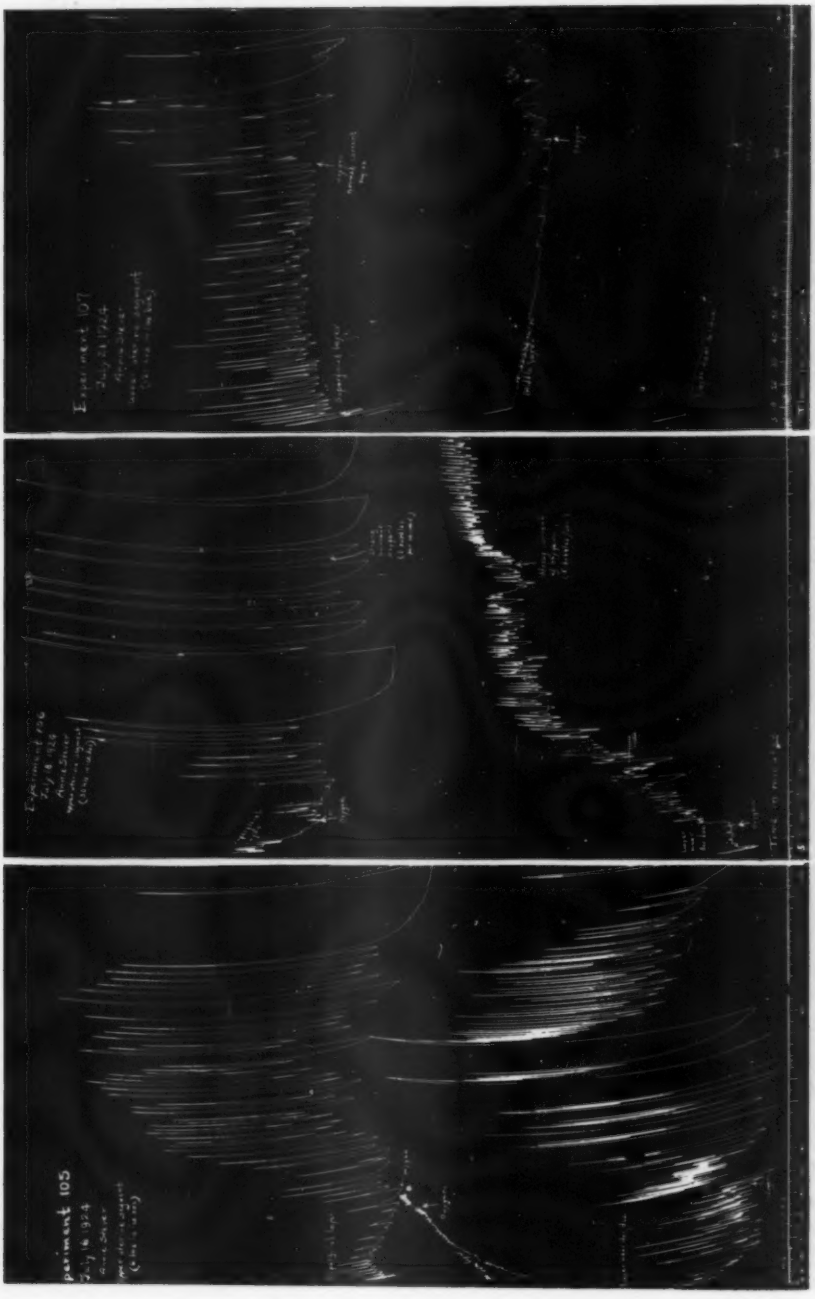

FIG. 12. SPONTANEOUS CONTRACTIONS AT DIFFERENT STAGES OF PRESERVATION

that agent in patients presenting mechanical disproportion. In that event, it would appear that both ends of the uterus attempt to expel the child, with the result that it escapes through its middle portion. Furthermore, the passive behavior of the lower segment makes it clear why hemorrhage continues in placenta praevia until the uterus has been completely emptied.

The observation that the contractions in the right half of the uterus are stronger that in the left may partially explain the frequent occurrence of right occipito-posterior presentations of the child, as well as the not unusual dextro-rotation of the uterus. Another interesting observation is the difference in rate and vigor of the contractions of the muscle from the several layers of the uterine wall. Our present state of knowledge concerning the normal arrangement of the fibres of the normal musculature is so imperfect that one is obliged to differentiate tentatively three layers in a specimen involving its entire thickness —a subperitoneal, a middle and a subdecidual layer. The last exhibits the most frequent and active contractions, the subperitoneal layer equally vigorous but less frequent ones, while the middle layer shows contractions of wide range. It seems probable that the activity of the subdecidual layer serves some other purpose than to foster the growth of the uterus or to expel the child; and it may be permissible to assume that such rhythmic contractions may serve as a "pumping mechanism," which has to do with the metabolism of the child—particularly in the supply of food and the elimination of waste material. Furthermore, after the expulsion of the afterbirth, it may exert a hemostatic action by compressing the vessels at the placental site and elsewhere in the decidua. Such a supposition is supported by the fact that these contractions soon disappear in the puerperal uterus, whereas in the case of secondary uterine inertia they persisted, while those of the subperitoneal and middle layers were in abeyance.

Strange to say, as has already been indicated, spontaneous contractions may occur in muscle which has been preserved for days in the ice-box, although they become progressively less vigorous with the age of the specimen. In this event, as shown in Figure 11, the contractions disappear first in the subdecidual layer.

Generally speaking, the intactness of a given specimen can be predicted by its appearance in the suspension fluid. If it appears cyanotic, it will still function; if it presents a pinkish appearance, it is dead, as is evidenced by extensive hemolysis. In an artificially produced tetanic state, as well as *in vivo* (see tracing of E. B. in Fig. 9), the contractions appear as slight humps, and present the same appearance as at the end of a long experiment.

In the puerperal uterus, no contractions occur in the subdecidual layer, and this may be interpreted as meaning that it has passed into a tetanic condition for purposes of hemostasis, or that it no longer functions as a "mechanical pump" in fostering the nutrition of the child.

On the other hand, the non-contractibility observed in the hemorrhagic areas from uteri opened on account of premature separation of the

normally implanted placenta has a totally different significance. In this event, the muscle fibres have been so damaged by the intramuscular infiltration with blood that they have lost all power of contraction.

SUMMARY

1. Spontaneous contractions of the musculature of the pregnant human uterus have been studied graphically at all periods of gestation, and arguing by analogy with the lower animals and by the fact that spontaneous contractions were observed in the uterus of a six-month foetus, it must be assumed that the human uterus contracts rhythmically throughout life, certainly from before birth until after the menopause.

2. In pregnancy the contractions differ in various individuals in the varying periods of pregnancy, labor and the puerperium. Likewise, differences are noted according to the portion of the uterus from which the specimen is obtained, as well as according to the length of time it has been preserved outside the body.

3. The characteristic, passive behavior of the lower uterine segment has been demonstrated.

4. The various observations made may be offered in support of many clinical intra-vitam phenomena, such as the Braxton Hicks contractions, the after pains, secondary uterine inertia, tetanic contraction of the uterus in prolonged labor, ligneous consistence of the uterus associated with premature separation of the placenta, and the frequent occurrence of right occipito-posterior positions of the child.

5. The presumable functions of the subdecidual layer of the uterine musculature have been discussed.

In conclusion I wish to express my obligations to Dr. Williams for suggesting this research, as well as for his constant interest and help during its prosecution. To the various members of the obstetrical staff I am under many obligations for kindly interest and assistance, as well as to Mrs. Caroline Pennington for photographing the kymographic tracings, and to Miss Elizabeth Phillips for preparing histological sections when necessary.

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Studies on the Physiological Anatomy of the Embryonic Heart

II. An Inquiry into the Development of the Heart Beat in Chick Embryos, Including the Development of Irritability to Electrical Stimulation

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PHYSIOLOGICAL studies on the hearts of embryos promise to shed much light on the functioning of the adult heart. It must not be forgotten, however, that progress in our knowledge of cardiac function must necessarily depend upon the correlation of the physiological data with an accurate knowledge of morphology. This is especially true in regard to the beating of the embryonic heart, for here also function is intimately concerned with structure. The studies on the developing heart have been largely morphological, but are still far from adequate to give a well rounded conception of its growth. For example, we know little about the exact time and manner of the differentiation of Purkinje tissue, nor are we thoroughly acquainted with the growth of nerves into the embryonic heart. Nevertheless, the way has been paved for a certain amount of physiological inquiry. As fortunately many of the morphological studies have been made on the chick heart, we have in the chick a favorable object for combined morphological and physiological investigation.

The earliest appearance of the heart is naturally associated with the development of the aorta. Recently, Dr. Sabin (1) has reinvestigated the manner of the formation of the earliest blood vessels and whence they come, and has been able to observe in the living chick the formation of the aorta. Prior to her study the manner of the formation of the aorta and the endocardium was not satisfactorily explained; by one group, the earliest vessels of the embryo were thought to come from an invasion of angioblasts from the embryonic membranes, following the idea of His, by another group these vessels were thought to arise *in situ*. By a modification of the McWhorter and Whipple (2) technique she was able to study in the living blastoderm of the chick embryo the formation of the blood vessels and the endocardium. In the embryonic membranes throughout the first two days of incubation Dr. Sabin observed a continual differentiation of mesenchymal cells into angioblasts; after the fifth somite stage a similar but independent differentiation of angioblasts occurred in the body of the

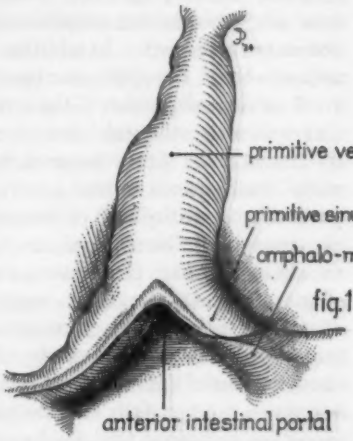
embryo itself. By further differentiation the angioblasts were seen to give rise to blood vessels. Hence the earliest blood vessels in the chick embryo, both in the blastoderm and within the embryo itself, arise *in situ* by the conversion of mesenchymal cells into angioblasts, clumps of which subsequently become canalized and transformed into primitive blood vessels. By the time of the formation of the ninth somite the chick possesses a definite double aorta which can be seen along the ventro-lateral margin of the somites, but the addition of angioblasts contributes to the growth of the aorta until the seventeenth somite stage.

The earliest stage in the formation of the endocardium was found by Dr. Sabin to be represented by isolated clumps of angioblasts which, by liquefying in their centers, form tiny vesicles between the bi-lateral anlage of the myo-epicardial mantle. From the walls of these vesicles sprouts of angioblasts are given off. These slender shoots join one another, enlarge, and then liquefy in the centers, so that a plexus is formed. By wide-spread cytolysis the plexus breaks down and eventually becomes converted into a single large vesicle. The wall of this vesicle is the primitive endocardium, enclosing the cavity of the primitive heart. The wall is formed of angioblasts which by further differentiation become endothelial cells. The fluid contained within the cavity is the product of cytolysis and represents part of the plasma of the developing blood. The original clusters of angioblasts which form the endocardium are directly continuous with, and indeed are a part of, the chain of those that form the aorta.

At some time between the appearance of the third and fifth somite the primordium of the myocardium makes its appearance. It is brought about by a thickening of the splanchnopleure of the amniocardiac vesicles. These two cardiogenic plates, so named by Mollier, one on the right side and one on the left side, fold around the forming endocardium and fuse when they meet in the mid-line. Both dorsal and ventral fusions occur cephalocaudally, so that the closure of the arterial end of the myo-epicardial mantle is completed before that of the venous end. With the fusion of the cardiogenic plates to form the myo-epicardial mantle, the heart is suspended from the ventral surface of the dorsal wall of the embryo by a double mesodermal membrane, called the dorsal mesocardium. In addition, there is a similar attachment from the ventral surface of the heart to the splanchnopleure anteriorly, called the ventral mesocardium.

By the time of the formation of the tenth somite the heart is a tubular structure, the cavity of which is lined with primitive endothelium. Cephalically, this chamber communicates with the lumen of the aorta. Caudally, it communicates with two veins, the omphalo-mesenteric vessels, which have arisen from angioblasts in a manner similar to that of the aorta and the chamber of the heart itself. These two veins become confluent at their junction with the primitive heart cavity. The myo-epicardial mantle meanwhile in its growth around the primitive heart has extended backward toward the confluence of these veins, so that now the heart consists of a myo-epicardium enclosing a tubular space in which two chambers may be

discerned, one formed by the confluence of the omphalo-mesenteric veins; the other, the original cavity of the primitive heart. The larger cavity is designated the primitive ventricle and is delimited cephalically by its connection with the aorta. Caudally, it joins the primitive sinus venosus, the chamber of the confluent omphalo-mesenteric vessels. The primitive



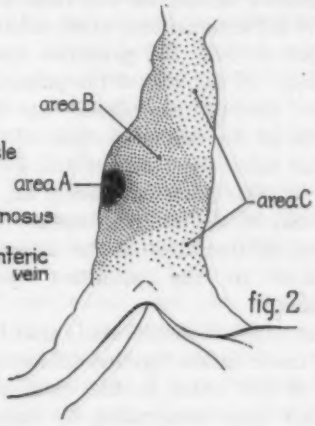


FIG. 1. Drawing of the heart of a ten-somite chick viewed from its ventral surface, showing the omphalo-mesenteric veins uniting with the tubular primitive ventricle. The bulging of the right side of the primitive ventricle is already visible at this stage. × 66.

FIG. 2. Outline drawing of the heart shown in Figure 1. The dark area (*A*) on the bulging greater curvature represents the region in which the first pulsations of the heart commence typically in the chick. From area *A* the beating portion spreads gradually along the entire right border of the heart and across the ventricle to the left border to include area *B*. The pulsations finally extend over the whole of the primitive ventricle to include area *C*.

sinus venosus is continuous cephalically with the ventricle, but is not yet sharply separated from it, while caudally it is continuous with the omphalo-mesenteric veins (Fig. 1).

The heart of the ten-somite stage is the starting point from which the chambers of the adult heart are derived. The bending to the right of the ventricle at this stage marks the beginning of a process of curving of the cardiac tube that is to continue until the heart assumes its permanent form at approximately the eighth day of development. Shortly after the cardiac tube has become curved to the right, it begins to undergo a change from a tubular into a segmented organ. This is brought about by a dilatation of the wall of the tube in three separate portions, so that the curved portion of the cardiac tube becomes converted into three chambers, the atrium, permanent ventricle and bulbus arteriosus (Fig. 3).

It is impossible at the present time to say exactly what portions of the originally unsegmented myo-epicardial mantle contribute to the formation of the walls of the atrium, permanent ventricle and bulbus arteriosus, respectively. At the ten-somite stage

the entire curved portion of the heart is undoubtedly the primitive ventricle, so that the myo-epicardium of the primitive ventricle is probably the primordium of the greater part of the walls of those chambers which arise from the curved portion of the cardiac tube. However, later in development, as the curved portion of the cardiac tube increases in length, it may very well receive myocardial cells from that part of the myocardium which extends backwards over the primitive sinus venosus. If the wall of the primitive sinus venosus contributes to the growth of the curved portion of the cardiac tube, these cells would go to form at least part, or possibly all, of the wall of the atrium, because the atrium differentiates as the adjacent chamber to the primitive sinus venosus.

The most exhaustive study that has been made on the regional differentiation of the heart in the chick has recently been undertaken by Patten (3). An examination of his figures shows what appears to be a downgrowth of myo-epicardial musculature from the primitive sinus venosus into the curved portion of the cardiac tube. The cephalic bulge of the left sinus horn seen in the ten-somite chick seems to have become incorporated into the curved portion of the cardiac tube by the sixteen-somite stage and takes the position of the future atrium.

The evidence would seem to show therefore that the permanent ventricle and bulbus arteriosus are derived from the myo-epicardium of the primitive ventricle, while the atrium is, at least in part, a derivative of the myo-epicardium of the primitive sinus venosus.

THE DEVELOPMENT OF THE HEART BEAT

Using a modification of the McWhorter and Whipple technique, Dr. Sabin (1) observed that the first movements of the heart of the embryonic chick occurred at the ten-somite stage. She also noted that the beating of the heart at this time is confined entirely to the primitive ventricle, whereas the primitive sinus venosus is quiescent. In addition, she noticed that the first contractions involved a small portion of the ventricular wall along the right margin near its venous end. From this area rhythmical contractions spread, involving gradually the entire wall of the ventricle, though the blood does not begin to circulate until the sixteen-somite stage. Recently, Dr. Sabin suggested that I continue this problem by making a more detailed study of the development of the heart beat; and she has given me her data and instructed me in the technique that she has used.

Previously to Dr. Sabin's observations the literature contains only scant mention of the development of the heart beat. Fano (4), in 1885, found that on the second day of incubation the heart of the embryonic chick is pulsating. Hooker (5), in 1911, referring to an unpublished observation by Chandler, states that the heart of the embryonic chick is beating at the eleven-somite stage. And Lillie (6), in 1908, mentions in his embryology of the chick that the heart commences to pulsate at the ten-somite stage. So far as the author is aware, these are the only references to the subject prior to the observations of Dr. Sabin.

Dr. Sabin used a technique whereby

the blastoderm is attached to a coverslip which is inverted upon a hanging-drop slide and sealed with vaseline. Since the whole blastoderm is studied intact, the heart with its connecting blood vessels and its covering membranes remains undisturbed. At the ten-somite stage, the heart can be seen very distinctly, in spite of the fact that the covering membranes enclose it. At a little later stage, however, the covering membranes begin to interfere noticeably with obtaining a clear view of the heart. Moreover, for physiological experimentation it is necessary to free the heart entirely from its membranes and have it exposed in a dish or the egg itself where it can be readily approached for manipulation. A method of this nature was therefore devised for the present observations. However, both Dr. Sabin's method of studying the blastoderm in a hanging-drop chamber and the new technique by which the heart is exposed by dissection have proved useful.

The latter method consists of opening an incubated egg, cutting out the blastoderm, and immersing it in a dish containing the blood serm of an adult hen. The blastoderm is passed through two changes of blood serum to remove the adherent yolk granules and the vitelline membrane. It is studied under a binocular microscope enclosed in a warm box. In order to anchor the blastoderm, it is advisable to fasten it to a cigarette paper by pressing the rim of the membrane against the paper with forceps; and if this is done so that the ventral surface lies upward, one has a beautiful view of the heart. To expose the heart for closer observation, it is necessary to dissect off the membranes which cover it, as well as to cut the ventral mesocardium. The removal of the ventral mesocardium requires care and practice, for moderate traction on this membrane often brings a beating heart to a standstill. The mesocardium, however, disappears in the course of development (eleventh somite), so that it forms an obstacle only in the earliest stages. A heart prepared in this manner will live for many hours, the longest experiment having lasted through a period of eight hours. By this method living chick embryos younger than the sixteen-somite stage can be advantageously studied.

For studying the first contractions of the heart, embryos of the nine-somite stage are necessary. As there is no exact correlation between the length of incubation and the number of somites, it is impossible to obtain the requisite somite stage without opening a large number of eggs; but as a rule a half dozen eggs of twenty-nine hours' incubation will yield one or two embryos of the nine-somite stage.

The first twitchings of the heart are confined entirely to the primitive ventricle and occur in a sharply localized area. This spot is on the right border of the ventricle about three-eighths of the distance from the venous to the arterial end, and represents only a very little of the myo-epicardial mantle. The location of this area is shown in the accompanying diagram (Fig. 2). From this spot the beat spreads and gradually involves more and more of the greater curvature of the primitive ventricle, spreading more rapidly toward the arterial than the venous end. When about three-fourths of this border is involved in the process of

contraction, the beating spreads to the lesser curvature. On the lesser curvature the contractile area first involves a point about one-third of the distance from the venous to the arterial end of the ventricle (Fig. 2). The beating then spreads along the lesser curvature in a manner similar to that in which it spreads along the greater curvature, until gradually the entire primitive ventricle is pulsating rhythmically. During this time there is absolutely no movement of the primitive sinus venosus. The first contractions of the myo-epicardial musculature along the greater curvature of the primitive ventricle occur at the ten-somite stage, and by the completion of the eleventh somite the entire primitive ventricle is pulsating rhythmically.

Although the primitive sinus venosus had as yet shown no rhythmical pulsation, the question arises as to whether or not the sinus, in spite of its being quiescent, inaugurates the stimulus for the beating ventricle, so in order to ascertain whether the contractions in the primitive ventricle are independent of any stimulus derived from the primitive sinus venosus, a ligature was tied at the junction of the primitive sinus venosus and the primitive ventricle. The technique for tying this ligature has been described in an earlier paper (7). This procedure produced no change in the beating of the primitive ventricle. This observation indicates that the "pacemaker" at this stage of development is not located in the primitive sinus venosus. Moreover, the sinus does not seem to acquire the power of inaugurating the heart beat until a period after the formation of the atrium, for the tying of ligatures at the sino-atrial junction soon after the formation of the atrium did not, in these experiments, produce heart block. It appears therefore, that the sinus does not inaugurate the rhythmical contraction of the heart until the end of the third or beginning of the fourth day—at the time when the sinus first begins to pulsate. For we have found that it is not until the fourth day of incubation, by which time the sinus, atrium, ventricle and bulbus arteriosus are all contracting in rhythmical sequence, that a ligature placed at the sino-atrial junction produces heart block. From our experiments it seems that the primitive sinus venosus is functionally undeveloped in the early heart, but that by the fourth day of development, perhaps coincidently with the first spontaneous pulsations of the sinus venosus, the power of acting as the "pacemaker" for the spontaneous muscular contractions of the heart shifts from the curved portion of the cardiac tube (atrium, ventricle) to the sinus.

We have described how a small area of the single-chambered primitive ventricle commences to pulsate and how this pulsation gradually spreads until it involves the entire ventricle, while as yet the primitive sinus venosus is quiescent. From this pulsating primitive ventricle, the atrium, the permanent ventricle and the bulbus arteriosus develop by the differentiation of three dilated segments in the ventricular tube (Fig. 3). The atrium, permanent ventricle and bulbus arteriosus beat consecutively from the time of their differentiation—a phenomenon of significance—as they are derived from the pulsating primitive ventricle. The atrium differentiates from that

portion of the myo-epicardial mantle of the primitive ventricle in which resides the "pacemaker." Hence the atrium in the course of its separation from the ventricle for a time remains the region in which the contractile impulse arises. As we have observed, by the fourth day of incubation the "pacemaker" has shifted finally from the atrium to the sinus venosus.

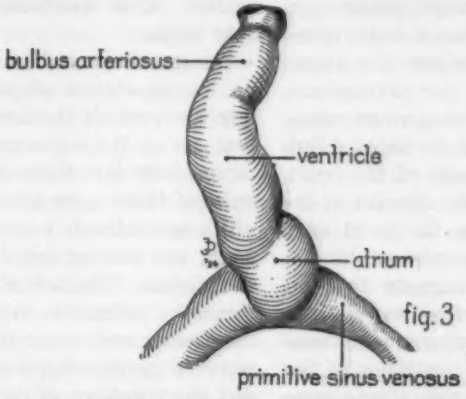


FIG. 3. Camera lucida drawing of the heart of a seventeen-somite chick viewed from the ventral surface, showing the differentiation of the primitive ventricle into atrium, ventricle and bulbus arteriosus. At this stage the atrium, ventricle and bulbus arteriosus are beating in sequence. The primitive sinus venosus has not yet begun to pulsate. × 66.

THE EFFECTS OF ELECTRICAL STIMULATION ON THE DEVELOPING HEART

In addition to studying the development of the heart beat observations have been made on the effects of stimulating the heart of the early chick embryo. The ages of the embryos used in these experiments ranged from twenty-nine hours' to five days' incubation inclusive. The period over which these observations range includes the stage before the inauguration of the heart beat (nine somites) up to and through the ingrowth of nerves from the central nervous system.

The method used for stimulating the heart consisted of unipolar excitation with mild induced currents. The stigmatic electrode was made out of a strip of tinsel with a tapering point of such delicacy that under a binocular microscope the point could be placed against the heart without producing damage. The fluid in which the embryo was immersed served as the indifferent electrode.

The manner of preparation of the embryo was determined by the age of the specimen. Chicks younger than sixteen somites were studied in a dish of blood serum under a binocular microscope enclosed in a warm box. Older embryos were studied in a well, built on the surface of the egg, into which the embryo was delivered according to the method described in an earlier paper[1] (7).

In addition to such electrical excitation of the heart after it had begun to

[1] Preliminary experiments on the effects of electrical stimulation on the heart were done in Dr. E. R. Clark's laboratory at the University of Georgia Medical School.

beat spontaneously, it was stimulated before any contractions had occurred in its musculature. It was thought that possibly there might be a period in development before spontaneous contractions occur, when stimulation of the cardiac tube might cause it to beat. Such a period would necessarily fall before the ten-somite stage.

In chicks, after the heart has begun to beat (ten-somite stage), traction on the mesocardium, when it is being removed in order to expose the heart, sometimes stretches the myo-epicardial mantle so as to bring about cessation of the beating of the heart. But before the development of the heart beat there is no such criterion of injury. So that when the heart of a nine-somite chick was exposed by dissection the only assurance that no injury had been caused was derived from the onset of spontaneous contractions during the differentiation of the tenth somite. As a rule, after a careful dissection under the experimental conditions here described, the heart begins to contract during this ten-somite stage.

In a number of instances the heart of the nine-somite stage, after careful removal of its covering membranes, was stimulated electrically with both make and break and tetanizing currents. In no instance has such a heart responded to stimulation by commencing to beat any sooner than it does normally (ten-somite stage). Similarly, in none of these experiments was such stimulation followed by even a single contraction of the heart musculature.

Furthermore, because the membranes covering the heart at this stage are exceedingly thin, and to obviate the possibility that their removal had so damaged the heart that subsequent response to electrical stimulation was impossible, several dozen hearts were stimulated with their membranes intact. In these experiments, too, the results were negative. Hence, it seems improbable that the heart can be induced by electrical stimulation to commence beating or to contract in any manner before the stage of ten somites, when spontaneous contractions begin.

The growth period from the ten- to the twenty-somite stage covers the time from which the heart begins to beat up to the segmentation of the cardiac tube into three chambers. A series of these more advanced hearts, which were already beating spontaneously, was also subjected to electrical stimulation. The lack of instrumental means by which the cardiac cycle in these early embryonic hearts can be recorded graphically, so that its rate and the character of its contractions can be absolutely determined, precludes the possibility of a statement at the present time that there is no effect whatever in the response of the early embryonic heart to electrical stimulation. But if there was any effect on the beating of the heart when any portion of the primitive ventricle or primitive sinus venosus was stimulated with either type of current, it was so slight that it could not be determined by inspection.

Still older hearts have been stimulated. For a considerable time after the differentiation of the atrium, permanent ventricle and bulbus arteriosus, stimulation of the heart in the manner just described did not alter the heart beat in any way in as far as could be determined by inspection.

Moreover, it was not possible to reverse the sequence of contraction of the secondary chambers of the early embryonic chick heart by stimulating either the bulbus arteriosus or the ventricle, so that it appeared that the myo-epicardial musculature of the early heart is not irritable to electrical stimulation.

At some time between the sixtieth and seventieth hour of incubation (thirty to thirty-five somites) the heart beat first begins to be noticeably modified when the heart is stimulated with the tetanizing current. By the seventieth hour of incubation stimulation of its venosus end with this current produced a most profound change in the character of the beating of the heart. While this part of the heart was being stimulated with this type of current, the rate of contraction of the atrium was so greatly accelerated that it was impossible to determine accurately the exact number of beats per minute by counting them under the binocular microscope. Moreover, it could be seen that the rate of contraction of the ventricle, as well as of the bulbus arteriosus, was accelerated following stimulation of the venous end of the heart. The rate of contraction of the ventricle and bulbus arteriosus ordinarily was found to be not synchronous with, but slower than, that of the atrium. Nevertheless the rate of contraction of the ventricle and bulbus arteriosus was increased often to triple that of the unstimulated heart. The rate of contraction of the ventricle and bulbus arteriosus, on the other hand, seemed synchronous.

When the venous end of the heart was stimulated with the tetanizing current for twenty to thirty seconds, the rate of the heart beat was profoundly accelerated during the period of stimulation. The moment that the stimulus was removed, instead of returning to its former rate of contraction, the heart beat ceased entirely for a short period. The length of this pause seemed to vary with the length of time during which the heart was stimulated. However, our method was inadequate to determine accurately the relationship between the duration of the stimulation and this compensatory pause. During this pause the heart was in diastole and all the chambers were filled with blood.

In addition to producing a marked increase in the rate of the heart beat followed by a compensatory pause, stimulation with a tetanizing current caused a change in the character of the contraction of the heart. The force and amplitude of contraction seemed modified. But our methods are as yet inadequate to analyze this change in the character of the contraction of the heart.

The youngest embryo in which we were able to produce any acceleration of the rate of the heart beat by electrical stimulation was of sixty-three hours' incubation. But it was only in embryos of seventy hours' incubation that a pronounced acceleration in the rate of the heart beat was obtained by electrical excitation. In chicks approximately of this age it was found necessary to stimulate the myo-epicardial musculature close to its venous end in order to produce the change. At a somewhat later stage (eighty to ninety hours' incubation) stimulation of any portion of the atrium with the tetanizing current affected the heart in the manner described. In chicks

less than seventy hours' incubation stimulation produced a moderate synchronous acceleration of the contraction rate of all the chambers, while stimulation of the venous end of the heart in chicks over seventy hours' incubation produced the phenomenon of asynchronous acceleration and the marked compensatory pause.

In addition to stimulating the atrium, the ventricle of chicks of about seventy hours' incubation was stimulated. The ventricle seldom responded to stimulation; but occasionally a tonic contraction of the musculature of this chamber resulted. When this occurred, the contraction persisted for a few seconds after the stimulation was stopped. These contractions represent the earliest response of the ventricle to electrical stimulation observed. During the period in which the ventricle showed this response, the atrium continued to beat uninterruptedly, whereas the bulbus arteriosus stopped beating. It was obvious, moreover, that in these experiments there was no sign of a reversal of the sequence of contraction of the chambers of the heart in response to ventricular stimulation.

In order to study the development of irritability of the ventricle to electrical stimulation this chamber was isolated from the atrium with a ligature and then stimulated. When the ventricle in a chick of eighty to ninety hours' incubation was stimulated close to the atrio-ventricular junction in the intact heart, the response was the same as if the atrium itself had been stimulated. However, when such a ligature was applied, the ventricle stopped beating temporarily and then resumed contracting at a slower rate than the atrium. After a ligature was applied at the atrio-ventricular junction the ventricle gave an entirely different response to stimulation, apparently because the stimulus could no longer spread to the adjacent atrium which behaved as "pacemaker" for the ventricle. In hearts prepared in such a manner it was not until between the ninetieth and one hundredth hour that the isolated ventricle responded to the tetanizing current by increase in the rate of its contraction. Stimulation produced an acceleration of the rate of contraction of the ventricle followed by a compensatory pause when the stimulus was interrupted. At approximately ninety to one hundred hours, also, the isolated ventricle was found to become irritable to the make and break currents, responding by individual contractions to this type of stimulation. These observations show that by the last part of the fourth or first part of the fifth day of development the ventricle is definitely irritable to electrical stimulation.

Moreover, it was in chicks of from ninety to one hundred hours' incubation, when the ventricle first responded to the tetanizing current by a definite increase in the rate of its contractions, that it first became possible to reverse the sequence of contraction of the atrium and ventricle in the unligated heart. If the atrium at this time was stimulated with a tetanizing current or with rapidly repeated makes and breaks for the period of a minute, the heart became quiescent on cessation of the stimulus and remained in diastole. During this period of rest, however, a single stimulation of the ventricle with either a make or break of the proper strength caused the ventricle to

contract. The contraction of the ventricle was followed immediately by almost simultaneous contractions of both the atrium and the bulbus arteriosus. This series of experiments afforded the earliest observed reversal of the sequence of contraction of the atrium and ventricle in the chick's heart.

The response of the bulbus arteriosus to electrical stimulation was also investigated. Stimulation of the bulbo-ventricular junction of the intact heart of chicks before eighty to ninety hours' incubation was demonstrated to produce no effect. In chicks of ninety to one hundred hours' incubation, at which time the ventricle has become independently irritable to stimulation, the tetanizing current, when applied to the bulbo-ventricular junction, accelerated the ventricle and bulbus arteriosus. That this response was really due to the excitation of the adjacent irritable ventricle could be shown by isolating the bulbus arteriosus from the ventricle with a ligature. After this procedure it was no longer possible to produce an increase in the rate of contraction of the bulbus arteriosus by stimulating it with the tetanizing current. However, at some time during the last half of the fifth day of incubation, the bulbus arteriosus apparently becomes independently irritable to the tetanizing current. At the same time this chamber seemed to become irritable to make and break stimuli.

These experiments seem to show that when the ventricle first responds to the tetanizing current by an increase in the rate of its contractions, it becomes possible to reverse the sequence of contraction of the atrium and ventricle. Similarly, during the last half of the fifth day of incubation when the bulbus arteriosus first responds to the tetanizing current by increase in rate of contraction, the sequence of contraction of the atrium, ventricle and bulbus arteriosus can be reversed. If, at this stage of development, the heart has been brought to a standstill immediately following stimulation of the atrium with a tetanizing current, reversal of the sequence of contraction of the atrium, ventricle and bulbus arteriosus may be accomplished by stimulating the bulbus arteriosus with the make or break current.

The only other findings which may be directly compared to these reported in the foregoing paragraph are those of Fano (4). This investigator, after finding that the spontaneous contractions of the heart commenced in the chick on the second day, stimulated the beating heart electrically and noted a resultant increase in irritability up to the sixth day of development. Between the sixth and tenth day, however, the response to such excitation decreased; after this stage the irritability was again increased. All of these observations of Fano were made without reference to the part of the heart involved and therefore furnish but scant data for direct comparison with the observations here reported.

SUMMARY

Continuing the observations of Dr. Sabin on the development of the heart beat in chick embryos, it was found that the first pulsations appeared at the ten-somite stage, and that the beat at this time was confined entirely to the primitive ventricle. Furthermore,

it was observed that the first spot in the primitive ventricle to beat was on the right border and occurred typically about three-eighths of the distance from the venous to the arterial end of the ventricle. The beating area then spread along the right border of the ventricle until gradually this entire border commenced to contract. When about three-fourths of this border had begun to beat, the contractile area had reached the left border of the ventricle. The area of contraction first involved the left border at a point about one-third of the distance from the venous to the arterial end of the ventricle. It then spread along the lesser curvature in a manner similar to its progression along the greater curvature, until gradually the entire primitive ventricle began to contract rhythmically. During the period when the heart beat was developing, and for a long time after the primitive ventricle was pulsating rhythmically, there was no movement of the primitive sinus venosus.

Moreover, it was shown that the primitive sinus venosus initially did not serve as the "pacemaker" for the pulsating primitive ventricle—a conclusion based on the observation that, when the primitive ventricle was isolated from the primitive sinus venosus with a ligature, no change was produced in the beating of the ventricle.

An account is given of how, as the primitive ventricle gradually gave rise to the atrium, permanent ventricle and bulbus arteriosus, by the twenty-somite stage, the atrium became the dominant rhythmical area of the heart. It was not possible to modify the beating of these chambers at this stage of development by separating them from the primitive sinus venosus with a ligature. By the fourth day of development, however, conditions were found to be changed: the isolation of the rest of the heart from the sinus venosus by ligation produced heart block. On the basis of these observations it seems apparent that the contractions of the primitive ventricle are independent of any stimulus derived from the primitive sinus venosus; but that at some time during the third day of incubation the "pacemaker" shifts from the atrium to the sinus venosus.

The earliest period in development at which the chick's heart could be definitely influenced by electrical stimulation was found to be at about the middle of the third day of incubation. We were not able to cause the heart to beat by stimulating it before spontaneous pulsation began at the ten-somite stage. Nor, even after the heart had begun to beat spontaneously, was it possible to influence its beating by electrical stimulation until long after the atrium, permanent ventricle and bulbus arteriosus had become functionally active. The first definite response to the electric current was obtained by stimulating the venous end of the heart in chicks of about sixty-three hours' incubation. This response consisted in a slight acceleration of the rate of the heart beat. By the close of the third day of incubation, stimulation of the venous end of the heart with the tetanizing current produced a most profound increase in the rate of contraction of the heart, which was followed by a compensatory pause when stimulation was stopped.

Furthermore, experiments are de-

scribed in which the atrium, ventricle and bulbus arteriosus were isolated from one another with ligatures and then stimulated individually. It was found that it was not possible to cause the isolated ventricle or bulbus arteriosus to respond to electrical stimulation by increase in rate of contraction until relatively long after the heart had begun to beat. The atrium became irritable during the last half of the third day, the ventricle by the last of the fourth or early part of the fifth day, and the bulbus arteriosus during the last half of the fifth day of incubation.

Further experiments showed that it was not possible to produce reversal of the sequence of contraction of the chambers of the heart by electrical stimulation until late in the fourth or the beginning of the fifth day of development. At this time, however, it became possible to produce reversal of the sequence of contraction of the chambers of the heart by stimulating the ventricle when the heart was brought into a state of compensatory pause following stimulation of the atrium by a tetanizing current. The phenomenon of reversal could be produced in the same way on the last half of the fifth day by stimulating the bulbus arteriosus.

One of the principal arguments advanced in favor of the myogenic theory of the beating of the adult heart is that it is possible to reverse the sequence of contraction of the chambers of the heart by electrical stimulation of the ventricles. As the present experiments demonstrate, it is, however, not possible to produce a reversal of the sequence of contraction of the chambers of the embryonic chick heart until long after the heart has commenced to pulsate spontaneously. The phenomenon of reversal cannot be demonstrated until the last part of the fourth or first part of the fifth day of incubation, which is the approximate stage in development when outgrowths of the central nervous system are definitely known to have entered the musculature of the heart.

It is a pleasure to express my appreciation to Dr. F. R. Sabin and Dr. G. B. Wislocki for the assistance they have rendered in the course of this study.

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Gynecology with Obstetrics. A Text-book for Students and Practitioners. By JOHN S. FAIRBAIRN, M.A., B.M., B.Ch. (Oxon.), F.R.C.P. (Lond.), F.R.C.S. (Eng.). 1924, 8°, 769 pages. $8.00.

Modern Aspects of Syphilis. By M. J. HORGAN, B.A., M.B., B.Ch., B.A.O., N.U.O. 1923, 12°, 136 pages. $1.75.

Common Infections of the Female Urethra and Cervix. By FRANK KIDD, M.A., M.Ch. (Cantab.), F.R.C.S. (Eng.) and A. MALCOLM SIMPSON, B.A., M.B., D.P.H. (Cantab.). With additional chapters by George T. Western, M.D. and M. S. Mayou, F.R.C.S. 1924, 8°, 194 pages. $2.50.

The Treatment of Fracture in General Practice. By C. MAX PAGE, D.S.O., M.S. (Lond.), F.R.C.S., and W. ROWLEY BRISTOW, M.B., B.S. (Lond.) F.R.C.S. 1923, 8°, 239 pages. $4.00.

Book Reviews

Basal Metabolism. By JOHN T. KING, JR. $2.50. (*The Williams & Wilkins Co., Baltimore,* 1924.)

The text of this book occupies 92 pages, to which are added selected tables and formulae commonly used in calculating or estimating the basal metabolic rate of children and adults of both sexes.

After a very brief historical introduction covering four pages there is a chapter on Physiology. Under that heading are discussed gas exchange, heat production, its relation to the thyroid, the "relation of the thyroid to other glands of internal secretion," normal standards of metabolic rates, and outlines describing the principles used in various methods—direct calorimetry and indirect determinations from the respiratory exchange. The influence on metabolic rates of thyroxin, epinephrin and several drugs are summarized under Pharmacology, and the caloric values of protein, carbohydrate and fat with their effect on the R.Q. are reviewed under the title of Chemistry. The last third of the book deals with basal metabolism in disease—which to the author is of primary importance in disorders of the thyroid. Five pages are devoted to the structure of this gland in relation to metabolic rates. A clinical review of hyper- and hypothyroidism follows, including comments on treatment, with changes in the metabolic rate as an index of its effectiveness. The text ends with the chapter "Metabolism in other conditions"—nutrition, oöphorectomy, skin conditions, pituitary disorders, diabetes, blood diseases, etc., a short paragraph being devoted to each. As to the significance of the test in connection with the thyroid there are these definite statements: "A basal metabolic rate of plus 15 per cent or higher is presumptive evidence of hyperthyroidism provided the other conditions that may increase the metabolism (leukemia, pernicious anemia, fevers, etc.) are absent." "There is no doubt that a reduced basal metabolism is the most constant recognizable feature of hypofunction of the thyroid gland."

The author has succeeded in his aim at presenting a brief survey of the literature and the value of basal metabolism in clinical medicine. Many of his discussions are excellent, though brief, such as the effect of giving oxygen inhalations to patients with anoxemia. As a whole, the book should be of use to students or to practitioners, but those who have some time to devote to the subject would probably prefer to consult more detailed reviews.

R. S. L.

Human Constitution. A Consideration of Its Relationship to Disease. By GEORGE DRAPER, M.D. 345 pp., 208 figs. $7.50. (*Philadelphia and London W. B. Saunders Co.,* 1924.)

Dr. Draper's book shows in its structure the effect of two important tendencies in present day medicine, the increasing emphasis on the constitutional factor in disease and the increasing use of numerical methods. By means of a careful and elaborate system of anthropological measurements he has studied the relationship of human morphology to gall-bladder disease, gastric and duodenal ulcer, pernicious anemia, asthma, nephritis and hypertension, and pulmonary tuberculosis.

Although the author disclaims any intention to state final beliefs, a word of caution may be useful to the non-statistical reader on the danger of conclusions based in many cases on a very small number of observations, in several instances as few as 7 to 9. This is the more necessary that no probable errors are to be found in the book. Means and probable errors of the different disease groups are given in the following table for two measurements, gonial angle, which is stated to be "one of the most constant,

reliable, and widely separated between the different disease groups," and umbilicus pubis-xiphoid umbilicus index, in which "there is a surprising uniformity in the values and definite massing of the indices according to diseases." The probable errors are calculated by the formula obtained by "Student" (*Biometrika*, VI, 12) for small samples:

$$\text{P. E. mean} = 0.67449 \frac{\sigma}{\sqrt{n-3}}$$

	GONIAL ANGLE MALES	INDEX FEMALES
Gall-bladder disease	112.16±0.98	71.7±1.5
Gastric and duodenal ulcer	121.94±0.81	80.1±2.9
Nephritis and hypertension	121.71±1.47	80.1±1.9
Pernicious anemia	127.27±0.94	
Tuberculosis	118.50±0.50	
Asthma		83.2±2.3

The differences of these means are as follows:

	DIFFERENCE		DIFF./P.E	
	Gonial angle	Index	Angle	Index
Tuberculosis—gall bladder	6.34±1.10		5.8	
Nephritis—gall bladder	9.55±1.77	8.4±2.4	5.4	3.5
Ulcer—gall bladder	9.78±1.27	8.4±3.3	7.7	2.5
Anemia—gall bladder	15.11±1.36		11.1	
Asthma—gall bladder		10.5±2.7		3.9
Nephritis—tuberculosis	3.21±1.55		2.1	
Ulcer—tuberculosis	3.44±0.95		3.6	
Anemia—tuberculosis	8.77±1.06		8.3	
Ulcer—nephritis	0.23±1.68	0.0±3.5	0.1	0.0
Anemia—nephritis	5.56±1.74		3.2	
Asthma—nephritis		2.1±3.0		0.7
Anemia—ulcer	5.33±1.24		4.3	
Asthma—ulcer		2.1±3.7		0.6

The gall-bladder patients are therefore significantly differentiated in gonial angle and probably in umbilical index from the other disease groups. In the former character the anemic are differentiated significantly from the tuberculous and ulcer cases and probably from the nephritic, while ulcer and tuberculosis are also probably differentiated from each other. The other differences are not significant.

It would be beyond the scope of this review to carry through a similar analysis of all the measurements dealt with. A comparison of the graphs for these two characters with the others will, however, give the reader at least a rough appreciation of the significance of the differences shown in the latter. In many of the characters we shall have to wait for more extensive data before we can distinguish constitutional differences from variations of sampling.

J. R. M.

Physical Diagnosis. By W. D. Rose, M.D. 750 pp. $8.50. (*St. Louis, The C. V. Mosby Company, 1924.*)

The fact that there have been four editions of this book, which was first published in 1917, testifies very definitely as to its popularity and general usage. Each edition has been an improvement, and the present one not only affords a useful textbook, but also supplies the general practitioner with a readable treatise on present day methods of clinical diagnosis. In hospitals as well as in medical schools, there is free access to various helpful laboratory procedures such as the X-ray and electrocardiograph—procedures which are more and more becoming available throughout the country. But, nevertheless, students should be taught self reliance and confidence in the ordinary "hand carried" methods of examining patients. They need a great deal of practice in seeing, feeling and hearing, and should be taught to exhaust their own resources before using those special laboratory examinations which are demanded in certain definite problems. It is quite probable, therefore, that students should be restricted to the ordinary procedures of physical diagnosis until their fourth year and that the third year should be the practice period for the use of their own hands. If such an idea be correct, it is not worth while to devote, in a book primarily for third year students, sections on radiographic and electrocardiographic diagnosis. On the other hand, a treatise on physical diagnosis cannot be too abundantly illustrated with pictures of pathological specimens. The dictum of Auenbrugger that sounds are to be explained by reference to corresponding physical states, and the life of Laennec equally divided between the clinic and the autopsy room, still make

necessary the association of pathological and clinical findings in the instruction of students. Physical diagnosis is a subdivision of clinical diagnosis, and perhaps this particular book tries to do too much and exceeds the boundary which is expressed by its title. Undoubtedly, there will be further editions of Dr. Rose's book. If criticism can sometimes be constructive, might it not be worth while to add more pathology and pathological physiology to the text and illustrations and restrict the size of the book by leaving out sections that more properly belong to clinical diagnosis?

E. W. B.

The Principles of Biochemistry. By THORBURN B. ROBERTSON. $8.50. (*Lea & Febiger, Philadelphia, Pa.*)

In the preface to the second (1924) edition of "Principles of Biochemistry," Robertson states that it has been his primary design to write a textbook for the use of medical students and students intending to specialize in biochemistry and physiology. This edition follows closely the scope and organization of the first edition, although new material has been incorporated.

To be of service to elementary students of a rapidly developing science, a text must be accurate, up to date, and balanced. Admirable as Robertson's book is in certain respects, one cannot but feel that it does not satisfy these three major requisites. The subject matter covered is so varied and so complex that it is impossible for any one person, writer or reviewer, to be familiar with the details of all phases. Those which I know at first hand, I have found described in a manner which is at times inaccurate and misleading. Much of the later literature (particularly the American literature), with modern points of view, is lacking.

It is always stimulating to read a text such as this, in which the author shows originality, and is not hide-bound by tradition concerning the proper distribution of space and emphasis among various divisions of the subject. Robertson clearly states that he wishes to present biochemistry in close relation to physiology. I would not quarrel with this idea were it not for the fact that much of the fundamental purely chemical material is hastily disposed of to make way for a discussion of physical chemical theories about which authorities differ radically, and also for a discussion of specialized phases of physiology, instead of general physiology. For this reason I say that the book lacks balance. Robertson's discussion of growth is of great interest but hardly compensates a student of biochemistry for the omission of much of the chemistry and physiology of the amino acids.

M. V. B.

Diphtheria, Its Bacteriology, Pathology and Immunology. By FREDERICK W. ANDREWES, WILLIAM BULLOCK, S. R. DOUGLAS, GEORGES DREYER, A. D. GARDNER, PAUL FILDES, J. C. G. LEDINGHAM AND C. G. L. WOLF. For the British Medical Research Council, 1923. Price, 12/6. (*His Majesty's Stationery Office, 1923.*)

This monograph is one of the most comprehensive that has been written on the subject of a single specific disease. For its completeness and balance the distinguished authors are to be congratulated. The attainment is made possible by the maturity of the subject itself. Our knowledge of the bacteriology, pathology and immunology of diphtheria has reached a stage that makes it possible and desirable that this knowledge be recorded in such a form.

The book throughout is well written. The introductory chapter on the History of Diphtheria is delightful. In the chapter on Diphtheria Antitoxin and Other Antibodies one misses Erlich's familiar diagrams, and in such a comprehensive work, which has an historical as well as a purely scientific value, it would seem that these diagrams should appear, although the reviewer is of the opinion that to a certain type of mind they are apt to be confusing rather than enlightening and serve to fix attention on a particular theory rather than on the facts involved. The Pathology of Experimental Diphtheria in Animals will be found very useful to research workers. The chapters on Laboratory Diagnosis, Serum Treatment, Immunity as a Factor in the Incidence and Spread of Diphtheria and The Prevention of Diphtheria are sane and should serve to place diagnosis, treatment

and prophylactic measures on a more rational basis than is often encountered. An adequate consideration of toxoid as an immunising antigen is lacking. The Virus as a Factor in the Incidence and Spread of Diphtheria is very well dealt with, as is also the final chapter on Diphtheroids. The very common pyogenic diphtheroid of animals, *C. pyogenes* (Glage) is barely mentioned in a table from Ebertson (1918), and is wrongly described as a non-fermenter of carbohydrates. The work of others who have studied this organism under the name of *Bacillus pyogenes* is entirely overlooked.

The illustrations, though not very numerous, are adequate and very good. The bibliography is very valuable, and the publishers as well as the authors are to be congratulated for the general form and convenience of the publication.

J. H. B.

How is Your Heart? By S. Calvin Smith, M.S., M.D. 208 pp. $1.75. (*Boni and Liveright, New York, 1924.*)

This little book consists of "intimate talks on the prevention of heart disease and on the care of the already damaged heart." Although one does not expect, of course, a critical discussion of the various factors causing heart disease, it should be pointed out that many of the views on this subject presented in this book rest on quite insecure evidence. The value of the description of the signs of heart disease is at least questionable; perhaps more harm than good might come of the advice on the determination (by the layman) of the character of his pulse, for example. The chief virtue of the book lies in the part devoted to a discussion of the care of the already damaged heart. These chapters are excellent and the instructions given therein are sound and clearly stated. On the whole, this little volume should serve a useful purpose.

W. H. R.

Duff House Papers. Volume 1. (Oxford Medical Publications.) Edited by Edmund I. Spriggs. $9.50. (*Henry Frowde; Hodder & Stoughton, London, 1922.*)

This attractively made volume of 380 pages brings together most of the scientific papers issued from Duff House (Banff) from its opening in 1913 to the date of publication. The bulk of the volume consists of papers reprinted from current journals, especially the *Quarterly Journal of Medicine.*

The new material consists chiefly of two papers: (1) A routine of radiographic examination of the alimentary tract, with special reference to the stomach and duodenum, by O. A. Marxer, an excellent comprehensive article profusely illustrated; and *The patients' manual of diabetes*, by E. I. Spriggs. This is printed as a separate volume for distribution to their patients. As this article antedates the use of insulin, much revision is already needed. However, there is much that should be helpful both to the intelligent patient and to the physician who has not had a wide experience in the management of such patients, particularly the elaborate instructions for the arrangement of diets, in minute detail, and the preparation of foods.

P. W. C.

The Pathology and Treatment of Diabetes Mellitus. By George Graham, M.A., M.D., F.R.C.P., First Assistant in the Medical Clinics, St. Bartholomew's Hospital; Physician, Royal Northern Hospital. $2.00. (*Oxford Medical Publications, London.*)

The Goulstonian Lectures on glycaemia and glycosuria, given by the author in 1921, form the basis for this volume. Some of his more recent work, as well as appendices containing diets, recipes, food values, chemical methods, and an explanation of "the aketogenic ratio" round the whole into a helpful little book on diabetes.

Part I, which deals with the physiology of the sugar metabolism, is simply and interestingly told. Part II, which contains an account of the different types of diabetes mellitus and the appropriate therapeutic procedure, suffers perhaps by being too simple. One cannot help feeling, however, that the author and his colleagues prefer conservative, steady methods to those of some other clinics, which are apparently more brilliant but which require more frequent and more radical changes. We

believe this type of work has a real place in the present day study of diabetes and are looking forward with interest to future reports promised by the same author.

H. M. T., Jr.

Materia Medica and Pharmacology for Nurses. By Gwendolen Hindes, M.Sc. 3/6 net. (*London, The Scientific Press, Ltd., 1924.*)

This is a little volume of 153 pages written by one who is a pharmacist and lecturer in materia medica and pharmacology, and who hopes "that it will be found useful by those (nurses) who have to take the new State Examination." The subject matter is very condensed, and there is careful selection of important points concerning the several drugs. But it is an English book and describes many preparations not in common use in the United States, while omitting some with which our nurses should be familiar. It does not offer help to the nurse in methods of the administration of medicines nor in the problems of percentage solutions and fractional doses. A survey of the book gives the impression that it would be more desirable as a review of the subject rather than as a text for the study of it.

L. K.

Materia Medica for Nurses. By A. L. Muirhead, M.D., and Edith P. Brodie, A.B., R.N. Second edition. $2.00. (*C. V. Mosby Company, St. Louis, Mo.*)

This is a textbook of 180 pages "written for the undergraduate nurse in training, with her needs and limitations constantly in mind." For the average school which devotes twenty-four hours to the subject of materia medica and where it is desired that the content of the course be fixed and the student knows just what is expected of her in the course, this little text fulfills its purpose admirably. The material is concise and presented in very good form with tracings to aid in visualizing the effects of some drugs. Especially in the chapters on solutions and the preparation of doses, the problems are very clearly explained, and confusion by giving several methods and verbose explanations is avoided.

Its chief fault is its briefness. It does not serve as a reference for a student who is called upon to give many of the newer remedies and who should have the stimulus and the opportunity to learn something about materia medica not only while studying in a few formal classes but daily whenever the occasion arises in her duties. It would seem more desirable to deduct from a text that is fuller than necessary for the immediate course than to be constantly supplementing.

L. E. K.

Manual of Psychiatry. By Paul E. Bowers, M.S., M.D. $3.50. (*W. B. Saunders Co., Philadelphia and London, 1924.*)

The author of this textbook claims that it gives "definite, detailed information" on psychiatry—a claim which is hardly substantiated either as to definition or detail. The introductory chapters are vague and sometimes misleading, and the clinical chapters are like summary re-echoes of another and better known treatise. It will be surprising if the student who reads the section on inheritance does not infer that manic-depressive insanity and dementia precox are proven to follow Mendelian laws, and also if he does not afterwards comfortably regard the "neuropathic constitution" as a unit character. The definitions in the chapter on symptoms are often obscure. To state that in clouding of consciousness, "the perceptions are faulty, owing to a dulling of perception" is a *circulus in definiendo*, which is not changed for the better by adding that it is a "defect in the mechanism of sensation which gives the brain but an imperfect visualisation of external stimulation." The definition of delirium fails to mention orientation. These are examples chosen at random. The neurological section includes similar curiosities of definition. "Agraphia," for example, "is due to forgetfulness on the part of the patient." The clinical chapters are better, but for the diagnosis of general paresis it is stated that the Wassermann reaction must be positive in the blood. Involutional melancholia is unhesitatingly said to belong to the manic-depressive group; and the prognosis is made to depend

directly on the physical condition. The assertion that in lethargic encephalitis (the old name is retained without explanation) periods of lucidity punctuate the state of lethargy suggests some obscurity in the author's mind as to the nature of the lethargic condition in this disease. The section on the relationship of insanity to crime is the best part of the book, probably because it contains the writer's specialized experience in this field. Dr. Bowers adheres throughout to the formal nosological type of psychiatry: and nowhere is there any evidence of the more comprehensive psycho-biological method. The book will aid the student to classify his patients, but it will not greatly help him to understand them.

R. D. G.

The Simple Carbohydrates and the Glucosides. By E. Franklin Armstrong, D.Sc., Ph.D. Fourth Edition. $5.00. (*Longmans, Green and Co., London, 1924.*)

This book, one of the Plimmer series of monographs on biochemistry, is valuable because it brings together and renders easily available the numerous recent developments in this highly complex and fundamental field. It is assumed that the reader has a general knowledge of the principles and methods of organic chemistry Although the treatise is not elementary, it is readable and comparatively non-technical. The treatment of the subject is essentially chemical, but sufficiently biological to make the monograph significant and interesting from this point of view also.

In scope and method of presentation the fourth edition is similar to the third. It treats of the chemistry of glucose and related compounds; the stereoisomerism of the carbohydrates; the relation between configuration and biological behavior; and the function of carbohydrates in plants. There is also a comprehensive bibliography. Of special interest is the discussion of the chemistry of the "new variety of glucose." An additional chapter has been introduced entitled "The Polysaccharides," which reviews the recent considerable addition to our knowledge of the structure of cellulose, starch, and inulin.

M. V. B.

Essentials of Surgery. By Archibald Leete McDonald, M.D. Second edition revised. $2.50. (*Philadelphia and London, J. B. Lippincott Company, 1923.*)

This is a very good companion for our old friend, *Essentials of Medicine* by Charles P. Emerson. It is a book of very nearly the same size, is gotten out by the same publishers and treats the subject of surgery in much the same manner as the other volume deals with medicine.

Bacteria and their connection with surgical conditions are reviewed, wounds in general and new growths are discussed, as well as general surgical anaesthesia and operations. Then, surgical diseases or conditions are treated under the various systems of the body. Gynecology and urology are included but not orthopedic surgery.

The book is intended primarily for nurses though it does not discuss technical nursing methods to any extent, but as its title indicates, it does cover the elements of surgery in such a way that the nurse may have a reasonably intelligent conception of surgical conditions.

L. K.

Gray's Anatomy, Twenty-first edition, 1924. Revised and reedited by Warren H. Lewis, M.D., Professor Physiological Anatomy, Johns Hopkins University, and Research Associate, Carnegie Institution of Washington. $10.50. (*Lea and Febiger, Philadelphia.*)

Into this edition of Gray's standard textbook of human anatomy Dr. Lewis has written a considerable amount of the new information made available by researches in pertinent fields since the last edition of six years ago. Much of the added material has to do with the development of the various organs, though many new facts of descriptive anatomy, particularly of the heart, lungs, sense organs and nervous system have also been incorporated. The chief addition in the field of anthropology consists of an interesting chart of comparative fetal measurements briefly but adequately explained. A series of sections through the arm and leg at various levels is a feature of the enlarged chapter on Surface Anatomy. Judicious use of figures from the original

papers serves to illustrate and clarify much of the new work. References to original sources are given whenever special mention is made of a particular work and a short general bibliography appears at the end of several chapters.

As in previous editions details are subordinated to coherence and brevity. Only the more common variations from the normal are given and these merely as facts, without serious attempt to explain the reasons for their occurrence. From a scientific point of view the volume could be enriched by a more detailed treatment of such embryological and comparative anatomical features as would give a more complete understanding and a real appreciation of both the normal and the "abnormal" definitive conditions. It is possible, however, that the practical ready reference value which the book now possesses might easily be diminished by encumbering it with such, necessarily voluminous, parenthetical material. As in the case of embryology, histological details are given only by way of introduction to the descriptions of the gross structure of the various organs. Brevity in this branch may be commended for two reasons; first, because of the manifest impossibility of complete and thorough treatment of microscopic structure in such limited space, and second, because in most medical schools the courses in gross anatomy and histology go hand in hand. In consideration of these facts, it is a debatable question whether such brief histological introductions as are given add materially to the value of the book.

No radical change in the arrangement of the subject matter has been made, and supplemented by the numerous added features, it remains what it has always been, a very valuable text for the student who desires his anatomical information presented in a brief, clear and easily accessible fashion.

F. H. S.

The Johns Hopkins Medical Research Club

Abstracts of Papers Presented

The Effect of Magnesium Ions on the Production of Protective Antibody Against the Pneumococcus. By WILLIAM A. PERLZWEIG. *From the Department of Medicine. Presented October 13, 1925.*

Madsen, Walbum and collaborators (for literature see Th. Madsen. *Jour. State Medicine*, 1923, xxxi, 1) have described able of inducing passive immunity represented by the protective antibody against the pneumococcus, the following experiment was carried out.

Five rabbits were each given intravenously a single dose of 1 cc. of pneumococcus type I vaccine, containing 33000 million heat killed organisms per 1 cc. of

	CONTROL RABBIT 1					CONTROL RABBIT 2					RABBIT 3					RABBIT 4					RABBIT 5				
Dilution of culture.....	$\frac{1}{10}$	$\frac{1}{10^2}$	$\frac{1}{10^3}$	$\frac{1}{10^4}$	$\frac{1}{10^5}$	$\frac{1}{10}$	$\frac{1}{10^2}$	$\frac{1}{10^3}$	$\frac{1}{10^4}$	$\frac{1}{10^5}$	$\frac{1}{10}$	$\frac{1}{10^2}$	$\frac{1}{10^3}$	$\frac{1}{10^4}$	$\frac{1}{10^5}$	$\frac{1}{10}$	$\frac{1}{10^2}$	$\frac{1}{10^3}$	$\frac{1}{10^4}$	$\frac{1}{10^5}$	$\frac{1}{10}$	$\frac{1}{10^2}$	$\frac{1}{10^3}$	$\frac{1}{10^4}$	$\frac{1}{10^5}$
February 28, '24.....	1 cc. pneumococcus type I vaccine intravenously to each rabbit																								
Date of serum																									
February 28, '24.....	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
March 5.....	S	D	D	D	D	S	S	S	D	D	S	S	S	D	D	S	D	D	D	D	S	D	D	D	D
March 10.....	S	D	S	D	D	S	D	D	D	D	S	S	S	D	D	S	S	D	D	D	S	D	D	D	D
March 17.....	S	D	D	D	D	D	D	D	D	D	S	D	D	D	D	S	D	D	D	D	D	D	D	D	D
March 17.....	Daily injections of NaCl begun, Rabbits 1 and 2										Daily injections of $MgCl_2$ begun, Rabbits 3, 4 and 5														
March 18.....	D	D	D	D	D	D	D	D	D	D	S	D	S	D	D	S	D	D	D	D	D	D	D	D	D
March 20.....	D	D	D	D	D	D	D	D	D	D	S	S	S	D	D	S	S	S	S	D	S	S	S	S	D
March 24.....	D	D	D	D	D	D	D	D	D	D	S	S	S	S	D	S	S	S	S	D	S	S	S	D	S
March 31.....	D	D	D	D	D	D	D	D	D	D	Rabbit 5		D			S	S	S	D	S	S	S	S	S	D
March 31.....	All salt injections discontinued										Salt injections discontinued														
April 2.....	D	D	D	D	D	D	D	D	D	D	–	–	–	–	–	D	D	D	D	D	D	D	D	D	D
April 14.....	D	D	D	D	D	D	D	D	D	D	–	–	–	–	–	D	D	D	D	D	D	D	D	D	D

D = mouse died within 72 hours and presence of organisms demonstrated by stained smear of peritoneal fluid.
S = mouse well and survived 5 days.
Each culture used was tested on unprotected mice and minimal lethal dose in each case was found to be 1×10^{-6} cc.

remarkable stimulating effects from the daily administration of various metal salts upon the elaboration of various immune substances (diphtheria antitoxin, agglutinins, opsonins, etc.) in immunized animals. Inasmuch as the published experiments of the Danish investigators did not include the effect of salts upon the elaboration of the type of substance cap-

0.8 per cent NaCl solution. The production of protective antibody in the blood of the rabbits was followed for 17 days by intraperitoneal injections into mice of 0.2 cc. portions of the sera mixed with graduated amounts of a virulent culture of type I pneumococcus. As shown in the table above, only very slight amounts of antibody were present in the rabbit sera

at the end of 17 days. Daily intravenous injections of 1 cc. of 0.001 molar $MgCl_2$ in 0.8 per cent NaCl solution per kilo body weight were then administered for 14 days to three of the rabbits (Nos. 3, 4 and 5). The other two rabbits (Nos. 1 and 2), serving as controls, received daily 1 cc. per kilo of 0.8 per cent NaCl solution intravenously. As seen from the table, the sera of the $MgCl_2$ treated rabbits showed a well marked antibody titer which disappeared when the administration of $MgCl_2$ was discontinued. The control, NaCl treated, rabbits showed a complete absence of protective antibody in their sera.

Studies on Respiratory Obstruction and Blood Flow in Dogs. By T. R. Harrison, A. Blalock and Charles P. Wilson. *From the Departments of Medicine and Surgery. Presented February 27, 1925.*

The effects of tracheal obstruction have been studied in dogs anaesthetized with morphine. The important findings were a diminished ventilation resulting in CO_2 retention and acidosis but not in anoxemia or in a diminution of oxygen consumption. There was a marked increase in minute blood flow without any significant change in pulse rate.

In a second series of experiments acid and alkali were injected into morphinized dogs. Acidosis resulted in an increased blood flow; alkalosis was associated with diminished blood flow.

These results are in accord with certain previous findings in patients with laryngeal œdema and asthma. No definite clinical conclusions can as yet be drawn. It is believed that morphia was an important factor in the results obtained.

Studies of the Cells of the Spinal Fluid and of the Meninges with Vital Methods. By Lawrence S. Kubie and Gertrude M. Schultz. *From the departments of Anatomy and Physiology. Presented January 16, 1925.*

The cells of the fluid and of the meninges have been studied in normal cats and after irritation with sub-arachnoid injections of trypan blue. The cells of the fluid have been counted differentially by the application of Sabin's methods of studying living blood cells. Scrapings from normal and meningitic arachnoids have been studied in the same way, and have also been grown as tissue cultures. Arachnoid spreads from fixed material have also been used, as well as sections from all regions of the nervous system. The following facts have become clear:

1. The initial response to an injection of trypan blue into the subarachnoid space is an outpouring of polymorphonuclear leucocytes. This is later supplanted by an increasing proportion of phagocytes which have the characteristics of both the monocyte and clasmatocyte of the studies of Sabin and Cunningham and Doan.

2. The small non-motile lymphocyte is the normal cell of the fluid and is found in increased numbers in all stages of irritation. It was found, however, that as the irritation is rendered chronic by repeated injections, the lymphocyte never becomes the most abundant cell in the first drops of fluid that issue from a needle placed in the cisterna; whereas the last drops that will flow are always predominantly lymphocytic. Furthermore, it has been shown that if, after the fluid has ceased to drop from the needle, an intravenous infusion of a hypotonic solution is begun, the resulting outpouring of fluid rises steadily in lymphocytic content. Sections from cats which are subjected to this procedure, when contrasted with controls, show that the increasing numbers of lymphocytes are due to their being washed out of the perivascular spaces of the brain and cord into the subarachnoid channels. The perivascular channels can be washed almost completely free of cells in this way. It has been shown on dogs that repeated subjection of the animal to this treatment is without apparent danger. The possible value of this attack on certain types of central nervous system disease is now under investigation.

3. The arachnoidal cell itself has been shown to be non-phagocytic. The macrophages of the meninges and of the fluid are seen to come from scattered phagocytes existing normally in the meninges and multiplying rapidly in response to irritation. In all cultures from normal or meningitic material these phagocytes can be seen forming a clear contrast to the arachnoid cell itself.

4. The application of these studies to human material is being begun, with findings to date which are consistent with these observations.

The Relation of the Sex Glands to Spontaneous Activity in the Rat. By CURT P. RICHTER. *From the Department of Psychiatry and the Henry Phipps Psychiatric Clinic. Presented February 27, 1925.*

This work is part of a series of experiments in which the relation between spontaneous activity and stimulation from the hollow viscera and the internal secretory glands is being studied. The present experiments deal only with the relation between the sex glands and activity. The basis for this work was a series of observations on the activity of the female rat made in the Psychological Laboratory by Dr. Ging Wang, using the revolving drum method. Wang found that the female rat shows a four-day cycle in activity, that is, it reaches a peak of activity every fourth day while on the three intervening days it is comparatively inactive. He showed by the vaginal smear method of Long and Evans that the peak of activity coincides with the period of oestrus which just precedes ovulation. Removal of the ovaries reduces spontaneous activity (about 95 per cent) to a flat low level free from the four-day cycle. The same results were obtained by Dr. Fred Allen on the male. Removal of the testes reduces the activity of the male to a flat low level which is very much the same as that of the spayed female. The activity of males and females may be differentiated by the presence of the four-day cycle in the female and by its absence in the male and by the higher running level of the female. The results of the foregoing experiments render it possible to make a quantitative as well as qualitative study of the effect of the transplantation of sex glands from one sex to the other.

In these experiments ovaries were transplanted to twenty-four castrated males. The ovaries were cut into small pieces and embedded in the recti muscles of the animals. In seventeen of the twenty-four animals there was an increase in activity to a new level which varied from 1.5 to 116 times as high as the pre-transplantation level. In seven animals there was no change at all. Thirteen animals showed the presence of cycles which were, however, not so regular as those of the normal female. Six of the animals reached a running level as high as that of the average female. Fourteen animals showed a definite change in body-weight following transplantation, that is, their body-weight became more like that of the female. Evidence was presented to show that the reduction in body-weight following the transplantation of the ovaries was open to a two-fold interpretation; either it could be partly due to the direct effect of the ovaries or partly due to the indirect effect of the increase in activity. Ovarian grafts were recovered from all of the animals which showed either a change in body-weight or an increase in activity. The ovarian tissue in these grafts was examined by Dr. Allan Gutmacher, who found that it was not normal but showed the presence of corpora lutea, mature follicles, ova and interstitial cells. Similar experiments in which testes are transplanted to spayed females are now being carried on.